WATER - HOLDING CAPACITY, LIPID OXIDATION, PIGMENT AND COLOR CHANGES IN RADIATION PASTEURIZED PREPACKAGED FRESH BEEF PRETREATED WITH SODIUM TRIPOLYPHOSPHATE

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ABSTRACT

WATER-HOLDING CAPACITY, LIPID OXIDATION, PIGMENT AND COLOR CHANGES IN RADIATION PASTEURIZED PREPACKAGED FRESH BEEF PRETREATED WITH SODIUM TRIPOLYPHOSPHATE

By Panfilo S. Belo, Jr.

This work was undertaken as an attempt to retard drip or exudate formation, lipid oxidation and oxidative discoloration during irradiation and storage of radiation pasteurized fresh beef through the combined use of sodium tripolyphosphate and vacuum packaging. Beef slices were dipped in sodium tripolyphosphate solution prior to vacuum packaging, irradiation and storage at 38°F.

Drip loss measurements, thiobarbituric acid tests, reflectance measurements, color and odor evaluations were conducted throughout a 21 day storage at 38°F. The effect of the subsequent exposure of vacuum packed beef samples to the atmosphere was also evaluated relative to lipid oxidation and oxidative discoloration. In general, phosphate pretreatment and vacuum packaging proved to compliment radiation pasteurization in the extension of the refrigerated shelf life of prepackaged fresh beef. While lipid oxidation was inhibited during storage in vacuum, phosphate pretreatment appeared to have effects on meats in addition to drip control. Water-holding capacity and color retention was improved during storage in vacuum and subsequent exposure to the atmosphere.

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INTRODUCTION

There is at present a growing realization that there is a need for centralized processing of retail cuts of fresh meat in order to reduce cost in cutting operations and in construction and equipment as well as to improve cutting operations. Such centralization however, requires much longer product life than what is now obtained. It appears that in order to obtain such centralization, means of preventing or retarding quality changes due to the processes associated with meat spoilage must be sought.

It has been proposed that wholesaling fresh meat based on radiation pasteurization would permit this centralization (Brownell et al., 1954). This method consists of preparing packaged standard cuts of meat in retail-size portions at a packing house rather than at the retail market. The packaged meat would be pasteurized at the packing house by means of a relatively low dose of ionizing radiation prior to shipping to the retailer.

Although pasteurizing doses of ionizing radiation (doses less than 1.0 Mrad) can prevent certain quality changes in fresh meat from occurring by destroying a large portion of microbial population, other changes not associated with microbial growth occur during storage. These changes include color changes, lipid oxidation,

formation or exudation of serum-like fluid commonly known as drip, textural changes and changes in odor and flavor (Coleby et al., 1960; Urbain, 1955, 1965). Such extended refrigerator shelf life afforded by radiation pasteurization can magnify this non-microbial degradation, thus offsetting the beneficial effect of radiation pasteurization.

This study was initiated as an attempt to retard the non-microbial degradations occurring during storage of radiation pasteurized fresh beef through the combined use of phosphate pretreatment and vacuum packaging. The entire process involves: (1) sodium tripolyphosphate pretreatment of beef slices to control drip formation and to improve water-holding capacity; (2) vacuum packaging to prevent lipid oxidation and retard the rapid oxidation of the heme pigments oxymyoglobin and myoglobin to brown ferric metmyoglobin; (3) low dose irradiation and refrigeration to control microbial spoilage and (4) exposure of the beef slices to the atmosphere to regenerate the red pigments prior to display. The effectiveness of the entire process was evaluated relative to drip formation, water-holding capacity, color changes and lipid oxidation after 21 days storage at 38°F. As the study progressed, however, the possibility of defining more fully the significance of phosphate treatment on the color of the product became apparent and was investigated.

REVIEW OF LITERATURE

Radiation Pasteurization

Since the early times, man has always sought means of preserving his food for future consumption. As technology advances, he has tried and applied new techniques of protecting his food from spoilage. In relatively recent years, a new approach to the problem of preventing microbial spoilage in foods has been developed. This involves treatment of the foodstuffs with ionizing radiation from electron or X-ray generators or from a radionuclide (Proctor et al., 1942; Brasch and Huber, 1947). Among the many types of ionizing radiations, only the high energy cathode rays, soft X-rays, and gamma rays find application in food preservation (Hannan, 1955).

The various biological applications of ionizing radiation have been conveniently grouped into various dose ranges depending upon the particular objective. A summary of the effects of different doses on various forms of life as described by Proctor (1959) is shown in Figure 1.

In food preservation, the application of radiation has been grouped into two broad categories: low-dose irradiation involving doses up to 1.0 Mrad and high dose irradiation involving doses above 1.0 Mrad (Brownell et al., 1954, 1955; Brownell, 1961; Heiligman, 1961). The former

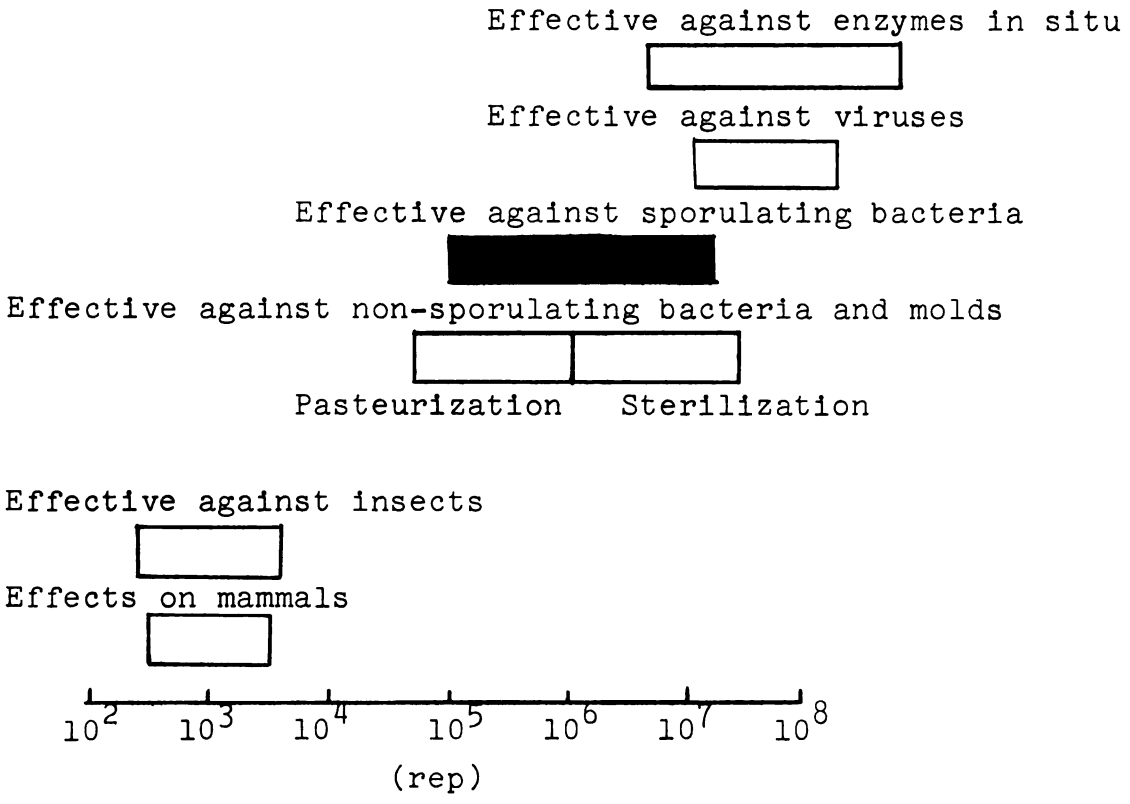

Fig. 1.--Dose levels of ionizing radiation required for various biological effects (Proctor, 1959).

is known as radiation pasteurization and the latter, radiation sterilization. Radiation doses from 1.0 to 5.0 Mrad have been applied to ham, bacon, chicken, beef and other meat and poultry products to accomplish "commercial" sterilization. As in thermal sterilization, it has the purpose of destroying all the microorganisms that might lead to spoilage of the product under normal storage conditions. On the other hand, pasteurizing doses of ionizing radiation (doses less than 1.0 Mrad) have been applied to luncheon meat, fresh meat and poultry, seafoods, fruits and vegetables. In this case, most but not all of the

microorganisms in the product are destroyed and their number is reduced to such an extent that the shelf life after treatment is substantially extended. The time taken for the surviving microorganisms to re-establish themselves in numbers which normally cause spoilage is greatly lengthened and hence, storage life can be extended.

According to Federal Food, Drug and Cosmetic Act of the United States Government, radiation is a food additive. Its use is regulated by the Food and Drug Administration and other government agencies. The regulation specifies a minimum and a maximum dose of the particular kinds of ionizing radiation used and the conditions carried out during the irradiation process. Presently, the use of radiation as a means of food preservation has been approved for two foods. They are: (1) potatoes, for sprout inhibition, and (2) wheat and wheat products, for insect disinfestation.

In the U.S.S.R., The Ministry of Health has approved still other products in addition to potatoes and grains. Official clearance has been given to the following products: fresh fruits and vegetables, raw meats, eviscerated chilled chicken, and culinary prepared meat products for reduction of microorganisms for shelf life extension; and onions for the purpose of sprout inhibition (Metlitskii et al., 1967). In Canada, clearance for the use of gamma radiation from cobalt-60 to inhibit sprouting in potatoes and onions has

been approved by the Food and Drug Directorate (MacQueen, 1966). The use of gamma radiation to inhibit sprouting in potatoes has also been approved in France and Israel.

Effects on Fresh Meat

The use of radiation pasteurization as a new food preservation process has been extensively examined. Its effect on microbial spoilage in meat has been carefully investigated (McLean and Sulzbacher, 1953; Wolin et al., 1957; Evans and Batzer, 1960; Rhodes, 1964; Hersom and Hulland, 1964).

The various chemical changes taking place in meat under the influence of pasteurizing doses of ionizing radiation have been reported by Wilson (1959), Obara and Ogasawara (1960) and Pal'min (1962).

The earlier works with radiation pasteurization of fresh meat suggested that the degree and character of various organoleptic changes depend on the dose, methods of treating the meat during irradiation and storage (Brownell et al., 1955; Schultz et al., 1956; Groninger et al., 1956; Doty et al., 1956).

In 1960, Lea et al. suggested that when beef is radiation pasteurized and stored in air, the maximum dose which does not cause significant loss of quality is likely to be below rather than above 100 Krad and that the deteriorative changes in fat may be the limiting factor. Heiligman (1961) on the other hand, reported that the

refrigerated (36° to 40°F) shelf life of vacuum packed raw beef steak can be extended to at least 16 weeks by treatment with low dose radiation (500 Krad).

Sokolov (1965) observed that it is difficult to detect changes in smell and taste in meat which has been exposed to 50 Krad. Changes in smell, taste and color are insignificant at doses 50 to 100 Krad, noticeable at 500 Krad and very pronounced at doses 1.0 to 2.0 Mrad.

The maximum dose of gamma radiation that can be applied to beef or lamb at 32°F in the absence of air, without causing changes in organoleptic qualities detectable by the trained panel, was reported by Rhodes and Shepherd (1966) to be 0.4 Mrad. They also reported that beef packed in vacuum and given 0.3 and 0.4 Mrad dose had normal meat odor after ten weeks at 32°F and showed no changes during the four days exposure to the air. Similar results were reported by Rhodes et al. (1967) in their studies on beef from barley fed animals.

Recently Urbain et al. (1968) reported that irradiation at 50 to 150 Krad in combination with phosphate treatment and vacuum packaging can extend the shelf life of prepackaged fresh beef to about 21 days at 38°F.

Water-Holding Capacity and Drip Formation in Fresh Meat

Water-holding or water-binding capacity is defined as the ability of the meat to hold fast to its own or

added water during application of any force such as pressing, heating, grinding and centrifugation (Hamm, 1960). This important property of meat has been shown to influence color, flavor, tenderness, juiciness and other meat qualities during all processing operations after slaughter (Arnold et al., 1956; Hamm, 1960; Lawrie, 1966).

In fresh meat the diminution of water holding capacity is manifested by the exudation or formation of serum like fluid commonly known as drip. This physical phenomenon is common in retail and prepackaged fresh meat cuts. Aside from being directly related to water-holding capacity, drip formation has been shown to be influenced by the area of the cut surface, the type of cuts, time of storage and temperature at which the fresh cut is kept (Hamm, 1960; Lawrie, 1966).

Use of Polyphosphates

It has been shown that incorporation of poly- or condensed phosphates into meats improve their water-holding capacity (Bendall, 1954; Mohler and Kiermeier, 1955; Swift and Ellis, 1956, 1957; Kamstra and Saffle, 1959; Sherman, 1961; Mahon, 1961). Investigations over the past decade indicate that phosphates merit a place in meat processing techniques. Different fields of application to meat and meat products have been recognized and these include: (1) cooked whole meat such as ham, picnics and bacon; (2) cooked comminuted meats such as sausages;

(3) poultry. The use of sodium tripolyphosphate in preventing drip formation in radiation pasteurized fish and shellfish has been reported by Spinelli et al. (1967).

Several postulates have been advanced to explain the nature of the action of polyphosphates in improving the water-holding capacity of meats. For instance, polyphosphates, due to their anionic nature, are believed to increase water-holding capacity through lowering of the isoelectric pH of muscle proteins (Hamm, 1960). Grau et al. (1953) and Hamm (1956, 1958b) advanced the theory that sequestering action of polyphosphate on calcium, magnesium and zinc increases the water-holding capacity of raw meats. Wierbicki et al. (1957a) demonstrated that calcium chloride and magnesium chloride increase fluid retention of beef heated at 70°C. Popp and Muhlbrecht (1958) however, take the view that polyphosphates do not increase the water absorption capacity of the meat. On the contrary, they merely restore the water absorption ability possessed before slaughter of the animal.

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. The increase of water binding is the result of the interaction between these and other fibrillar proteins. Yasui et al. (1964) showed that there are two types of phosphate bindings of polyphosphate to myosin.

The first of these is a direct binding of highly polymerized polyphosphate such as hexametaphosphate 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 ions, whereas the first is inhibited under this condition. 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 are not bound, polyphosphates increase the ionic strength and solubilized the proteins.

Effect of Ionizing Radiation

Changes in water-holding capacity of meat due to ionizing radiation have been observed by various workers. Groninger et al. (1956) showed that there was a slight increase in expressed drip in meat due to irradiation from 0.6 to 15 Mrad. Noticeable loss in water-holding capacity in meat proteins was reported by Cain et al. (1958). Kuprianoff (1957) also noted that irradiation often results in loss of juice and he theorized that this effect is due to structural changes in muscle proteins.

Loss of fluid as drip was among the changes observed by Rhodes and Shepherd (1966) during the extended storage of radiation pasteurized lamb sides and beef joints. The amount ranged from 0 to 5% of the weight of the meat. As a consequence of the exudation, part of the soluble pigment

in the drip was transferred to the surface layers of the fat and gave a faint pinkish tinge. It is, however, uncertain whether this fluid was exuded as an inevitable consequence of the post-mortem changes or was induced by the application of pasteurizing doses of ionizing radiation.

Methods of Determination

Most of the methods used in the determination of water-holding capacity of meat are based on the measurement of water liberated by application of pressure on the muscle tissue. These include sedimentation method (Mohler and Kiermier, 1953a), centrifugation (Janicki and Walczack, 1954a; Kormendy and Gantner, 1954; Hamm, 1958b; Wiebicki, et al., 1957a; Sherman, 1961), pressing method (Grau and Hamm, 1953; Wismer-Pedersen, 1958; Wierbicki, 1958) and ultracentrifugation (Lloyd and Morgan, 1933). The exact amount of loosely bound water in meat cannot be determined by any of these methods. Meat contains various protein components and the water of hydration of each is not known and besides the amount of physically absorbed water is changed by the various laboratory methods. The relative changes in water holding capacity, however, can be determined by considering the various muscle proteins as a single component and by using the same method under the same experimental conditions (Wierbicki and Deatherage, 1958).

Lipid Oxidation and Pigment Changes in Gamma Irradiated Meat

Meat undergoes oxidative deterioration during exposure to the atmosphere and radiation. The two types of oxidative changes which occur in meat are the oxidation of fats and of heme pigments. Oxidation of fats is manifested by the formation of rancid odor and flavor whereas oxidation of heme pigments causes discoloration. These two processes have been shown to be inter-related and in fact they can accelerate one another (Watts, 1954). Together they constitute a large part in the spoilage of fresh meat especially in prepackaged or retail cuts.

Lipid Oxidation

The formation of peroxides in lipid during irradiation had been demonstrated (Mead, 1952; Hannan and Shepherd, 1954; Poling et al., 1955; Chipault et al., 1958). The radiation-induced oxidation in meat has been reported to be affected by total dose, dose rate, presence or absence of oxygen during irradiation and post-irradiation storage, presence of free radial acceptors and antioxidants, and temperature during irradiation and storage (Chipault, 1962).

In 1960, Lea et al. reported that a dose of 100 Krad greatly accelerated lipid oxidation in beef at chilled storage, while at 50 Krad, the effect was variable but always appreciable. Samples irradiated at 25 Krad

however, showed signs of oxidation only after a very long storage.

The reduction of preformed lipid peroxides by radiation in inert atmosphere and anaerobic packaging has been reported (Goldblith and Proctor, 1955; Groninger et al., 1956; Lea et al., 1960; Greene and Watts, in press).

Methods of evaluating oxidative changes.--The degree to which fats in meat and meat products have undergone oxidation has been evaluated by means of several chemical and physical methods. These include peroxide value determination (Lea, 1939), Kreis test (Patton et al., 1951), manometric measurements of oxygen uptake (Tappel et al., 1961), and thiobarbituric acid test (Sinnhuber et al., 1958; Tarladgis et al., 1960; Evans, 1961). Organoleptic evaluation of rancidity in meat has also been carried out by several investigators (Timms and Watts, 1958; Tarladgis et al., 1962; Greene and Watts, in press).

Oxidative Discoloration

Meat has a purplish color immediately after slaughter. Upon exposure to the atmosphere, it quickly assumes a bright red color and longer exposure turns it to brown. These color changes have been shown to be caused principally by the reaction of myoglobin with atmospheric oxygen (Hill, 1933; George and Stratman, 1952; Watts, 1954; Landrock and Wallace, 1955; Fox, 1966).

The color cycle which occurs in fresh meat have been fully elucidated. The cycle is shown in Figure 2. The cycle starts with myoglobin which is a purplish compound

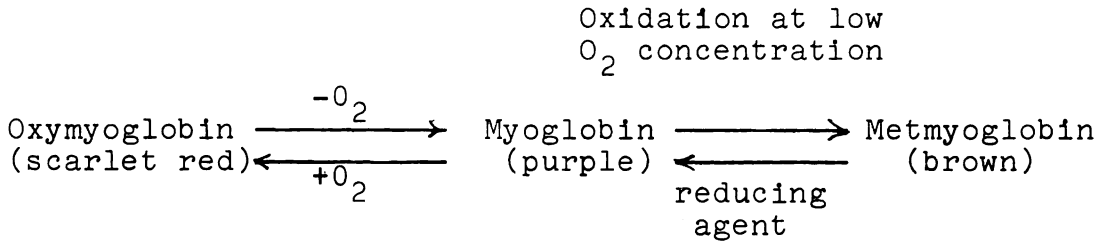

Fig. 2.--Color cycle in fresh beef (Landrock and Wallace, 1955).

and in which the iron in the phorphyrin ring of the heme protein is in the ferrous state. This type of color is normally predominant in uncut meats. Upon exposure to air, oxygen attaches to heme protein forming a scarlet red compound, oxymyoglobin. This reaction is an oxygenation and is reversible depending upon the concentration of oxygen. If the oxygen supply is cut off as in vacuum packaging, the oxymyoglobin is transformed to myoglobin. Myoglobin in turn can be oxidized to a brown compound, metmyoglobin in which the iron in the phorphyrin ring of the heme protein is in the ferric state. This reaction takes place if the oxygen concentration is low. The metmyoglobin can be transformed to myoglobin by means of reducing agents and by enzymatic reduction. Enzymatic reduction of metmyoglobin in anaerobically packed meat has

been observed by Walters and Taylor (1963) and Cutaia and Ordal (1964).

Several factors have been reported to influence metmyoglobin discoloration. Niell and Hasting (1925) were the first to observe that the rate of oxidation of the heme pigments was accelerated at low partial pressure of oxygen. Later, Brooks (1935) and George and Stratman (1952) reported that at 1 to 20 mm Hg partial pressure of oxygen, there was a maximum rate of oxidation of heme pigments. The rate at these pressures, however, was dependent on the concentration of the pigment, pH and temperature. Landrock and Wallace (1955) reported that oxygen permeability of packaging films affects the rate of oxidative discoloration of prepackaged fresh meat. They further showed that with oxygen-impermeable film, the metabolic process involved in pigment change would eventually stop for lack of oxygen. At this condition the heme pigments are in fully reduced myoglobin state. Upon re-exposure to the atmosphere, the reduced myoglobin would oxygenate and the meat would "bloom."

The role of pH in the metmyoglobin formation has been reported by Cutaia and Ordal (1964) and Stewart et al. (1965). Their results agreed in that as the pH of the meat decreases (from 5.95 to 5.45) the rate of metmyoglobin conversion to myoglobin decreases. This effect was attributed to the effect of pH upon the enzyme systems causing the generation of reducing power.

Stewart et al. (1965) are of the opinion that metmyoglobin formed during storage is a result of two opposing factors. These factors are autoxidation of the ferrous pigment to metmyoglobin on one hand and enzymatic reduction on the other hand. The net result of these two reactions is determined by the various factors as pH, temperature and other environmental factors.

Meats irradiated with ionizing radiations often undergo pronounced color changes. These color changes have been noted and reported by many investigators (Ginger et al. 1955; Groninger et al., 1956; Schweigert et al., 1955; Tappel, 1956). It is generally observed that meat irradiated in excess oxygen undergoes brown discoloration with formation of metmyoglobin either immediately after irradiation or upon subsequent storage. With high irradiation doses, the hematin compounds may be destroyed oxidatively to a large extent. In the practical range of irradiation (0.046 to 2.8 Mrad) according to Tappel (1956), these oxidative changes can be avoided by irradiation in inert atmosphere or high vacuum.

Lea et al. (1960) and Rhodes and Shepherd (1966) reported that pasteurizing doses of ionizing radiation greatly accelerated oxidation of heme pigments under aerobic conditions. Similar results were reported by Rhodes et al. (1967) in their studies on irradiated beef from barley fed animals.

Methods for investigation of color changes in meat.--Measurement of the amounts of the various forms of myoglobin present at the surface of the meat have been carried out in several ways. Broumand et al. (1958) applied absorption spectrophotometry to extracts of meat taken from the surface of beef cuts. Winkler (1939a) and Winkler et al. (1940) described an objective method for following color changes on meat surface using a photoelectric comparator. A rapid non-destructive technique for measuring the relative concentration of oxymyoglobin, myoglobin and metmyoglobin at the surface of the meat based on reflectance spectrophotometry has been proposed by Dean and Ball (1960). Stewart et al. (1965), Snyder (1965), and Snyder and Armstrong (1967).

Reflectance spectrophotometry as applied to meat is based on the fact that the reflectivity of the three major pigments (myoglobin, oxymyoglobin and metmyoglobin) is the same at a single point at 525 mμ. This point which is known as the isosbestic point of the three pigments, can be used as a reference for total pigment content (Snyder, 1965; Stewart et al., 1965). Myoglobin and oxymyoglobin are isobestic at 571 mμ while 474 mμ is the isosbestic point for oxymyoglobin and metmyoglobin. Using the ratio of the isosbestic point at any two pigments to the isosbestic point of the three pigments, the relative concentration of any pigment can be obtained. For instance, the

ratio of the reflectivity at 571 mμ to that at 525 mμ will give the relative amount of metmyoglobin while the ratio at 474 mμ to that at 525 mμ will measure the relative amount of myoglobin in the total pigment present at the meat surface.

Coupled Oxidation of Heme Pigments and Lipids in Meat

Haurowitz et al. (1941) showed that free radical intermediates from lipid oxidation can decompose hemes causing loss of color. On the other hand, evidence was shown by Younathan and Watts (1960) and Brown et al. (1963) that during oxidation of oxymyoglobin and myoglobin ferric heme pigments can catalyze the oxidation of the tissue lipids in meat.

It appears that vacuum packaging is the most feasible method to use in conjunction with radiation pasteurization. One disadvantage of vacuum packaging, however, is that reduced myoglobin which is purple predominates on the meat surface. This is not the typical color the consumer associates with fresh meat.

The use of phosphate seems to offer a solution to the existing problem of drip formation in prepackaged fresh meat. Phosphate may also have a beneficial effect on the color of the meat.

By using phosphate, vacuum packaging, irradiation and subsequent exposure of the meat to the atmosphere, a

solution to the existing problem of prepackaged fresh meat salable life extension is anticipated.

MATERIALS AND METHODS

Meat

Rounds from U.S.D.A. "Commercial" grade round were used throughout these experiments. The beef was freshly cut from the intact rounds of unknown history received at the meat department of Michigan State University Food Stores. Further cutting and preparations of the meat samples were done at the Food Science Laboratory.

Phosphate Pretreatment

Phosphate pretreatment was carried out by dipping meat samples into phosphate solutions. Sodium tripolyphosphate was obtained from Calgon Corporation, Pittsburg, Pennsylvania. Preparations of dipping solutions are based on percentage weight aqueous solution.

Packaging Materials

Packaging materials used include:

1. Laminate ("L" pouches) consisting of polyester (Mylar) base with a thin coat of polyvinylidine chloride (saran) applied to the outer surface and a heavier extrusion coat of polyethylene on the inner surface which acts as a sealant. These highly gas-impermeable pouches were supplied by the International Kenfield Distributing

Company under the trade name "IKD Super All-Vak #13." The size of the pouches used was 7" x 8".

2. Plasticized polyvinyl chloride fresh meat wrap film "Resinite RMF-61" manufactured by the Borden Chemical Company. This is a highly oxygen-impermeable film.

Package sealing is accomplished by the Kenfield package sealer (Model C-14) which has a vacuum and gas flush attachments.

Methods of Irradiation

Irradiation was conducted using the pool-type Co-60 gamma source housed in the Food Science Building, Michigan State University, East Lansing, Michigan. The strength of the source was measured to be 51,000 curies in May, 1967. The dose rate at 50 cm from the source center was measured to be about 200 Krad per hour.

The source is comprised of 24 BNL MK-1 doubly encapsulated source strips arranged in a cylindrical pattern and when the source is in the fully up-position, are enclosed in a stainless safety shield having an outside diameter of 34.5 cm. The time required to raise the source from the bottom of the pool to the fully-up position was 19-21 seconds. The pool depth is 15 feet.

Packaged meat samples which were mounted on cardboard supports were placed at measured distances from the source center and exposed to gamma radiation for a predetermined

length of time. The time and distances were based on doses previously measured by Fricke chemical dosimeter.

Drip Measurements

In the determination of the optimum phosphate concentration and optimum dipping time that will effectively retard drip formation, the method of Howard (1956) for drip measurement was used. Beef slices having a dimension of 5 x 5 x 1 cm (shortest dimension being in the direction of the muscle fiber) were dipped in phosphate solution, drained and placed in tared aluminum dishes approximately 8 cm in diameter and 5 cm deep. In each aluminum dish were placed glass grids which served to keep the samples out of contact with the exuded fluid. Sample and container were weighed immediately and sealed with cellophane tape to prevent moisture loss through vaporization. Samples were then stored in the refrigerator maintained at 38°F. Periodic determinations of drip loss throughout the storage period were made by measuring the 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 percentage drip loss.

For retail size samples used in the study on the effect of various pasteurizing doses of ionizing radiation on drip formation, an alternative method was used. Samples were packed in moisture-impermeable film, vacuum sealed, irradiated and stored in the refrigerator. Loss

of weight was determined at the end of storage by draining the samples on a wire screen. The drip loss was calculated as loss of weight.

Phosphate Absorption Determination

The amount of sodium tripolyphosphate solution that was absorbed by the meat was determined by weighing the samples before and after dipping and draining.

Analyses for total phosphorus content of both treated and untreated samples were made to determine the amount of sodium tripolyphosphate that was absorbed by the meat. In the analyses, meat samples were ground and phosphorus was determined on aliquots by colorimetric method of the Association of Official Agricultural Chemists (1960).

pH Measurements

The pH of the meat was measured before and after phosphate pretreatment, immediately after irradiation, during storage and at the end of 21 days. A 1:10 mixture of the meat sample and distilled water was blended in a Waring Blendor. The pH of the blended mixture was determined with a Beckman Model 76 Expandometric pH meter.

Water-holding Capacity Determination

The centrifugation method by Sherman (1961) for the determination of percentage water retention was used.

Meat samples were ground by passing three times through a laboratory-size mincer fitted with 3-mm plate. Ten grams of meat were weighed into a tared centrifuge tube (50 ml capacity) and 10.0 ml of distilled water was introduced from a burette. After thorough mixing with a glass rod, the tubes were stoppered and the mixture was allowed to stand overnight for 18 to 24 hours at 38°F. The stopper was removed and the tube was centrifuged at 3000 rpm for 20 minutes in a Lourdes Model AX centrifuge. Afterwards, the supernatant fluid was decanted through a presoaked and drained filter paper resting in a glass funnel into a 10 ml graduated cylinder that could be read to within 0.05 ml. The mixture was drained into the filter paper for 10 minutes. The water-holding capacity of the sample expressed as percentage water retention per gram of meat was calculated by the equation given as follows:

$$\frac{\text{Vol. (ml) fluid added - vol. (ml) fluid not absorbed}}{\text{Weight (gm) of meat}} \times 100$$

Lipid Oxidation Measurements

Lipid oxidation was measured by thiobarbituric acid (TBA) test (Tarladgis et al., 1960). A ten gram meat sample was blended with 50 ml of distilled water in a Waring Blendor for two minutes. The mixture was transferred quantitatively into a Kjeldahl flask by washing

with an additional 47.5 ml distilled water. The pH of the mixture was adjusted to about 1.5 by adding 2.5 ml concentrated HCl. A small amount of Dow Silicone defoamer was placed into the lower neck of the flask and the mixture was heated on the Kjeldahl distillation apparatus. Using the highest heat obtainable on the distillation apparatus, 30 ml distillate were collected within 10 minutes from the time boiling begins.

A 5-ml aliquot of the distillate was pipetted into a glass stoppered tube and 5 ml of 0.02 M 2-thiobarbituric acid in 90% glacial acetic acid solution were added. After mixing the contents, the tube was immersed in a boiling water bath for 35 minutes. A distilled water-TBA reagent blank was prepared and treated the same way as the samples.

After heating, the samples were cooled in tap water for 10 minutes. A portion of the solution was transferred to a colorimeter test tube and the optical density was read against the blank at wavelength of 538 mμ. A Bausch and Lomb Spectronic 20 spectrophotometer was used.

Results are expressed as TBA number based on malonaldehyde standard curve. The standard curve was prepared by making appropriate dilutions of 1×10^{-3}M 1, 1, 3, 3-tetra-ethoxy-propane standard solutions to give amounts ranging from 5×10^{-8} to 2×10^{-7} moles malonaldehyde in 5 ml. Determination were run directly

on 5 ml by the method described above for testing the distillate.

The TBA number, expressed as milligrams malonaldehyde per 1000 grams of meat, was calculated based on the 68% distillation recovery as reported by Tarladgis et al. (1961).

Measurement of Pigments

Visual observations of meat samples were made during storage in vacuum and during exposure to the atmosphere. Subsequently, samples were prepared for reflectance measurements. The method used to determine the relative amount of pigments at the surface of the meat was reflectance spectrophotometry as described by Snyder (1965) and Stewart et al. (1965). A Beckman D.U. spectrophotometer with reflectance attachment was used. Samples were contained in plastic ice cube holders of dimension great enough to cover the light port of the reflectance attachment. A freshly scraped block of Mg) was used as a reference standard.

Reflectance readings were obtained on the absorbancy scale at 571 mμ, 525 mμ and 474 mμ. The readings were next converted to reflectance values for infinitely thick samples or reflectivity value (R_∞) using standard tables which relate absorbance to percentage transmittance. Reflectivity values (R_∞) were in turn converted to K/S values using Table D in the appendix of Judd and Wyzecki (1964). K is the absorption coefficient and S is the

scattering coefficient. These values account for the fact that a fraction of light is scattered by the opaque meat surface, and that the proportion of the light absorbed by the pigment to the scatterd light from the sample decreases with increasing reflectivity.

The ratio of K/S at 474 mμ and K/S at 571 mμ to that at 525 mμ were obtained. The ratio $\frac{\text{K/S at 571 mμ}}{\text{K/S at 525 mμ}}$ was used to determine the relative amount of metmyoglobin by referring to a standard curve.

To develop a standard curve, reflectance measurements were taken with meat samples in 100% myoglobin, 100% oxymyoglobin and 100% metmyoglobin. Preparations of these samples were done as follows:

Samples of 1 3/4 x 1 1/2 x 3/4 inch dimensions were prepared from the ribeye (semitendinosus) muscle of "Commercial" grade beef bottom round and placed in plastic ice cube holders. The samples were immediately vacuum packed in oxygen-impermeable pouches (allowing them to bloom as little as possible) and left at room temperature for four hours. This time period was enough to obtain samples having a predominantly myoglobin pigment as indicated by the constant reflectance spectra. The samples were then measured as representing 100% myoglobin.

The samples were then unwrapped, left for two hours at 38°F, packaged in oxygen permeable film (Resinite RMF-61) and stored overnight at 38°F. The reflectance of the

samples was measured and was considered to represent that of 100% myoglobin.

The same samples were unwrapped and the surface was painted with 1.0% $K_3Fe(CN)_6$ at one hour intervals. The samples were then overwrapped with Resinite RMF-61 film and stored overnight at 38°F. The reflectance of the samples was measured the following day and was considered to represent that of 100% metmyoglobin.

Reflectance readings were obtained at 571 mμ, 525 mμ and 474 mμ against a reference standard MgO. Readings were obtained on the absorbancy scale and then converted to reflectivity value (R_∞). Reflectivity values were in turn converted to K/S values. The ratio of K/S at 474 mμ and K/S at 571 mμ to that at 525 mμ was obtained.

An assumed linear curve between K/S ratio at 571 mμ/ 525 mμ for 0 and 100% metmyoglobin was constructed. This curve served as standard curve in the determination of the relative proportion of metmyoglobin at the surface of the samples.

Organoleptic Evaluation (Color and Odor)

Meat samples which have been stored for 20 days in vacuum were rewrapped with oxygen-permeable film and exposed to the atmosphere for one day at 38°F. Samples were then presented to a panel consisting of 10 to 12 judges. The panel was asked to rate the color and odor of the meat on the following scale: poor, very bad, bad,

fairly bad, marginal, fairly good, good, very good and excellent. Numerical values of 1 to 9 (from poor to excellent) were assigned and the average sensory scores were calculated.

RESULTS

Effect of Various Concentrations of Sodium Tripolyphosphate Dipping Solution and Dipping Time on Drip Loss and Phosphate Absorption in Fresh Beef

The first part of this study was designed to determine the optimum concentration of sodium tripolyphosphate dipping solution and the optimum dipping time that will effectively retard drip formation in beef slices during storage at 38°F for 21 days. The amount of sodium tripolyphosphate that was absorbed by the meat was also determined. Beef slices were prepared from ribeye muscles of "Commercial" grade round. The meat was trimmed of adipose tissue and sliced into samples with a dimension of 5 x 5 x 1 cm. Samples were cut in such a way that the shortest dimension is in the direction of the muscle fiber.

The ribeye muscle from a "Commercial" grade round was selected since it is convenient to prepare uniform size samples and there is some indication that cuts from the ribeye muscle are more sensitive to drip than cuts from other parts of the carcass. The pattern of cutting the samples is based on the findings that the amount of drip from small samples is greater when the shortest dimension (i.e., its thickness) is along the fibers of

the muscle than when it is across them (Howard, 1956).

In the determination of the optimum concentration of dipping solution that will effectively retard drip formation, samples were dipped in 2.5, 5.0, 7.5 and 10.0% (per cent by weight) aqueous solution of sodium tripolyphosphate for 30 seconds and drained on a wire screen for about 10 to 15 minutes. The temperature of the dipping solution was about 72°F. Samples that were not dipped were run along as control. The samples were then placed in aluminum dishes and stored at 38°F. Periodic determination of the amount of drip formed during the entire storage period was conducted using the method previously described. Three identical experiments were conducted at different times with different pieces of meat and the average of nine measurements per treatment is shown in Figure 3. It is shown that drip formation was effectively prevented to a greater degree when the samples were dipped in 7.5% and 10.0% aqueous solution of sodium tripolyphosphate than when they were dipped in 2.5% and 5.0% solutions. Samples which did not have any pretreatment showed considerable amount of drip loss during the 21 days storage.

To determine the optimum dipping time that will effectively retard drip formation, samples were dipped in 10.0% aqueous solution of sodium tripolyphosphate for

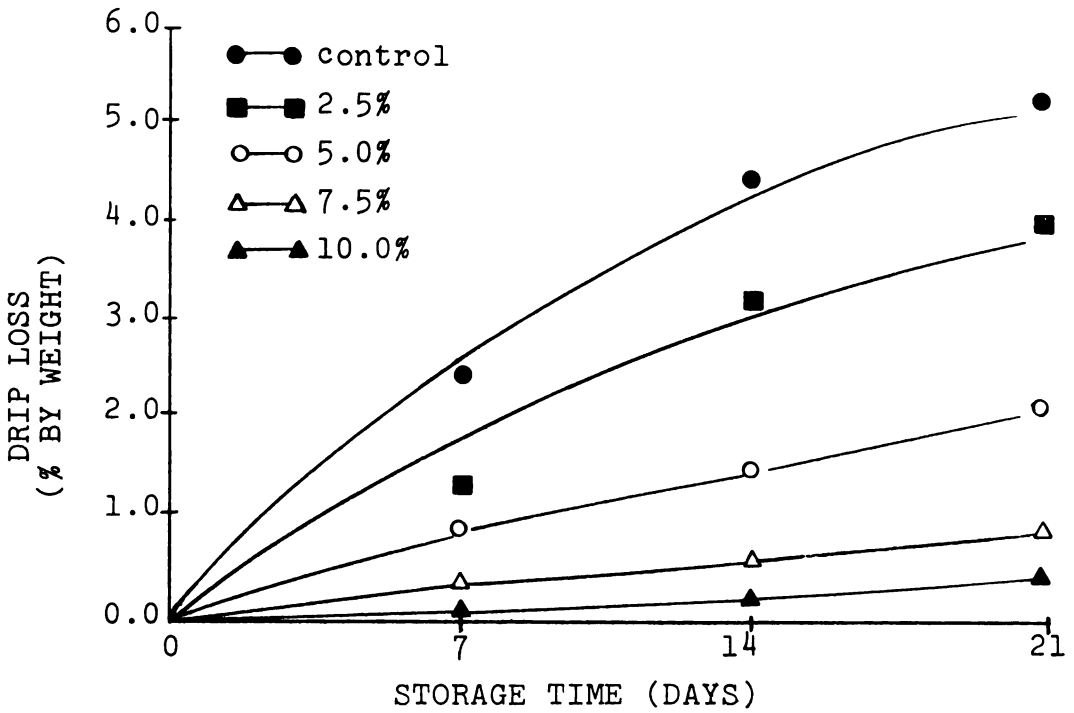

Figure 3.--Drip loss during storage at 38°F of fresh beef slices dipped at various concentrations of sodium tripolyphosphate for 30 seconds.

0, 10, 20, 30, 60 and 120 seconds. The samples were drained and stored at 38°F for 21 days. Periodic determination of drip was conducted throughout the storage period.

The amount of sodium tripolyphosphate absorbed by the meat at various dipping time was determined by total phosphorus analysis before and after dipping and draining. Figure 4 shows the results. Dipping from 30 to 120 seconds resulted in a significant retardation of drip formation throughout the entire 21 day storage at 38°F. The amount of sodium tripolyphosphate absorbed by the meat shows a rapid increase from 10 to 60 seconds dipping time. This, however, tends to level off from 60 to 120 seconds.

It is apparent that dipping times from 30 to 120 seconds in 10.0% sodium tripolyphsophate can effectively retard drip formation up to 21 days at 38°F. Phosphate uptake determinations at various dipping times showed a mean sodium tripolyphosphate uptake of 0.43% at 30 seconds, 0.53% at 60 seconds and 0.57% at 120 seconds.

Effect of Phosphate Pretreatment on Drip Formation, pH and Water-Holding Capacity of Radiation Pasteurized Fresh Beef

The following experiments tested the effectiveness of phosphate pretreatment in retarding drip formation in radiation pasteurized beef slices stored in vacuum at 38°F for 21 days. Beef ribeye muscles from "Commercial" grade round were trimmed of adipose tissue and sliced

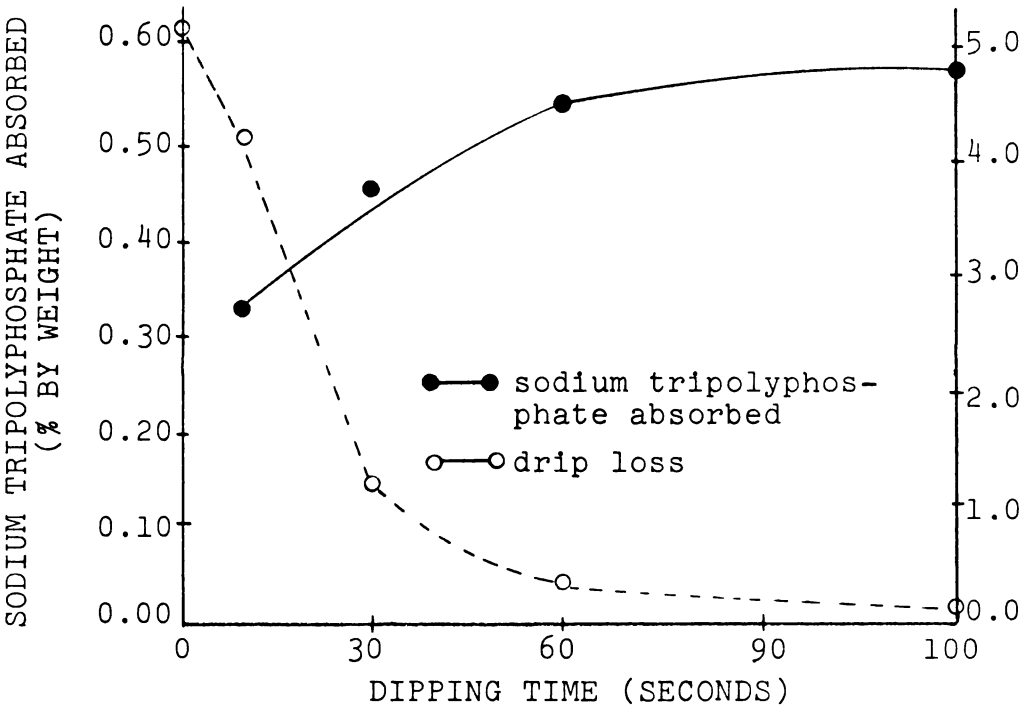

Figure 4.--Phosphate uptake by fresh beef slices dipped for various time intervals in 10.0% sodium tripolyphosphate and drip loss after three weeks storage at 38°F.

into 1 to 2 cm thick samples. The weight of the samples ranged from 100 to 250 grams. After weighing, the samples were dipped in 10.0% aqueous solution of sodium tripolyphosphate for 30 to 60 seconds. The temperature and pH of the dipping solution were about 72°F and 9.5 respectively. The samples were next drained on a wire screen for 10-15 minutes, weighed and vacuum packed in oxygen-impermeable pouches (IKD-Super A110Vak #13). They were mounted on cardboard supports and irradiated at 0, 50, 100, 150, 250 and 500 Krad. Samples which did not have phosphate treatment were irradiated at the same dosages. After irradiation they were dismounted from the supports and stored at 38°F for 21 days.

The amount of sodium tripolyphosphate solution that was absorbed by the meat and pH changes during the dipping process were determined. Table 1 gives these results. The amount of solution uptake is shown to vary between meat samples used in different runs. The amount of solution uptake by various meat samples ranged from 0.91 to 2.00 per cent by weight. In all cases, the treatment process caused an increase in the pH of the meat. The increase in the pH ranged from 0.37 to 0.45 pH unit.

Data in Figure 5 show that in spite of the increase in weight due to the dipping process, much less percentage drip loss was observed on the treated samples than on the control. There appeared to be no noticeable change in the amount of drip formed as caused by the various

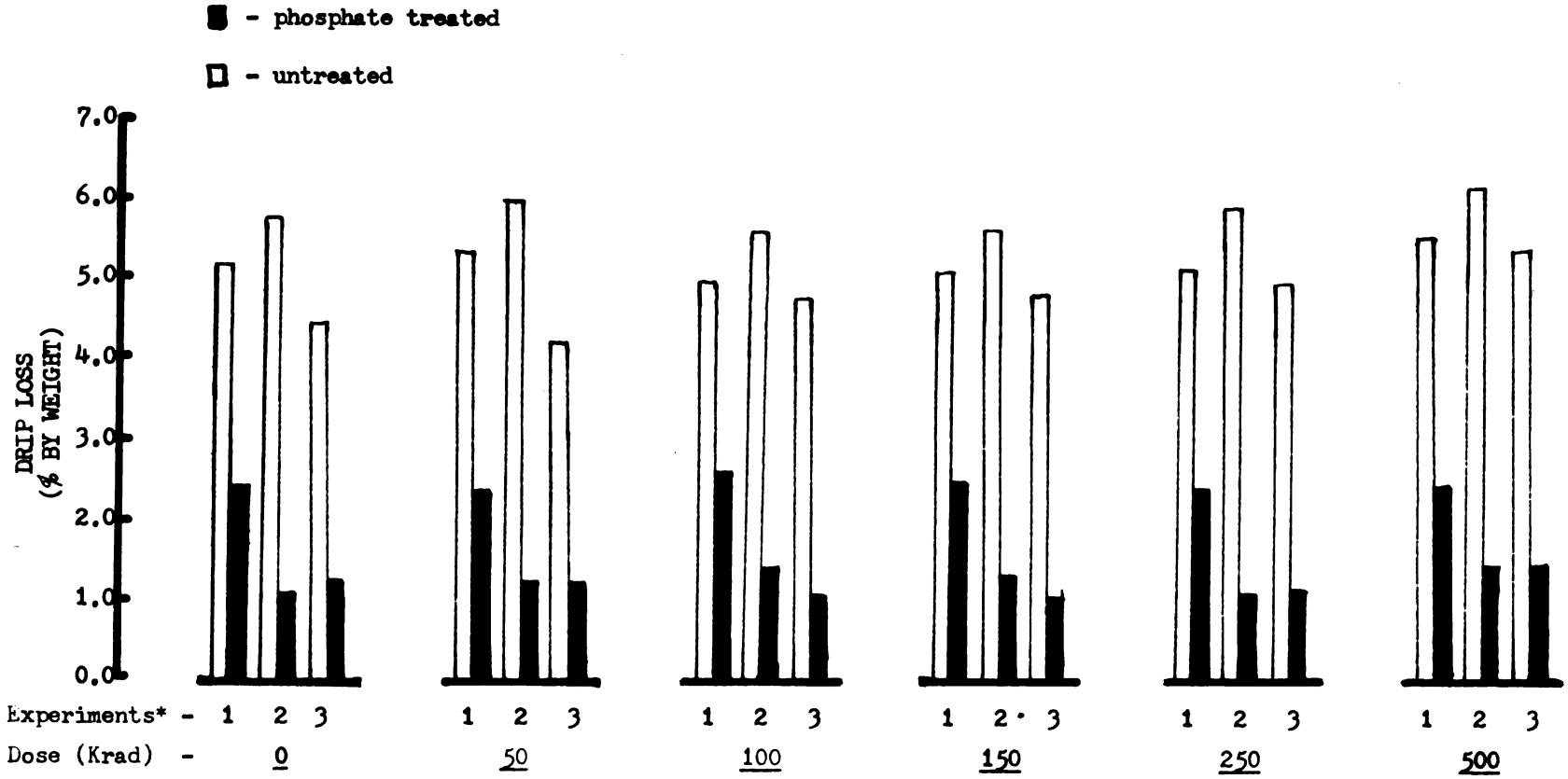

Figure 5.--Loss of drip in fresh beef slices that were vacuum packed, irradiated at various pasteurizing doses of gamma radiation and then stored for 21 days at 38°F. The drip in meat that was treated with sodium tripolyphosphate solution prior to irradiation is compared with the drip in beef slices that were not treated.

* - Identical experiments performed on different days with different pieces of meat.

TABLE 1.--Solution uptake and pH of fresh beef slices after dipping in 10.0% solution of sodium tripolyphosphate for 30 to 60 seconds and draining for 10 to 15 minutes.

Experiment*	pH Before Dipping	pH After Dipping	% Solution Uptake**
1	5.50	5.95	1.00-1.62
2	5.60	6.03	0.91-1.62
3	5.75	6.12	1.14-2.00

*Identical experiments performed at different times and on different pieces of meat.

**Range obtained from 18 samples (per cent by weight).

pasteurizing doses of ionizing radiation. There was, however, a slight increase in drip loss due to vacuum in both phosphate treated and the untreated samples.

Figure 6 shows the percentage water retention of irradiated meat samples after 21 days of storage at 38°F. It is shown that phosphate pretreatment greatly increased the water holding-capacity of the beef samples. The increase in the percentage water retention due to phosphate pretreatment ranged from 6.40 to 9.00%. The percentage water retention or water holding capacity is shown to differ among samples used in different runs. No large changes in percentage water retention due to pasteurizing doses of gamma radiation were observed.

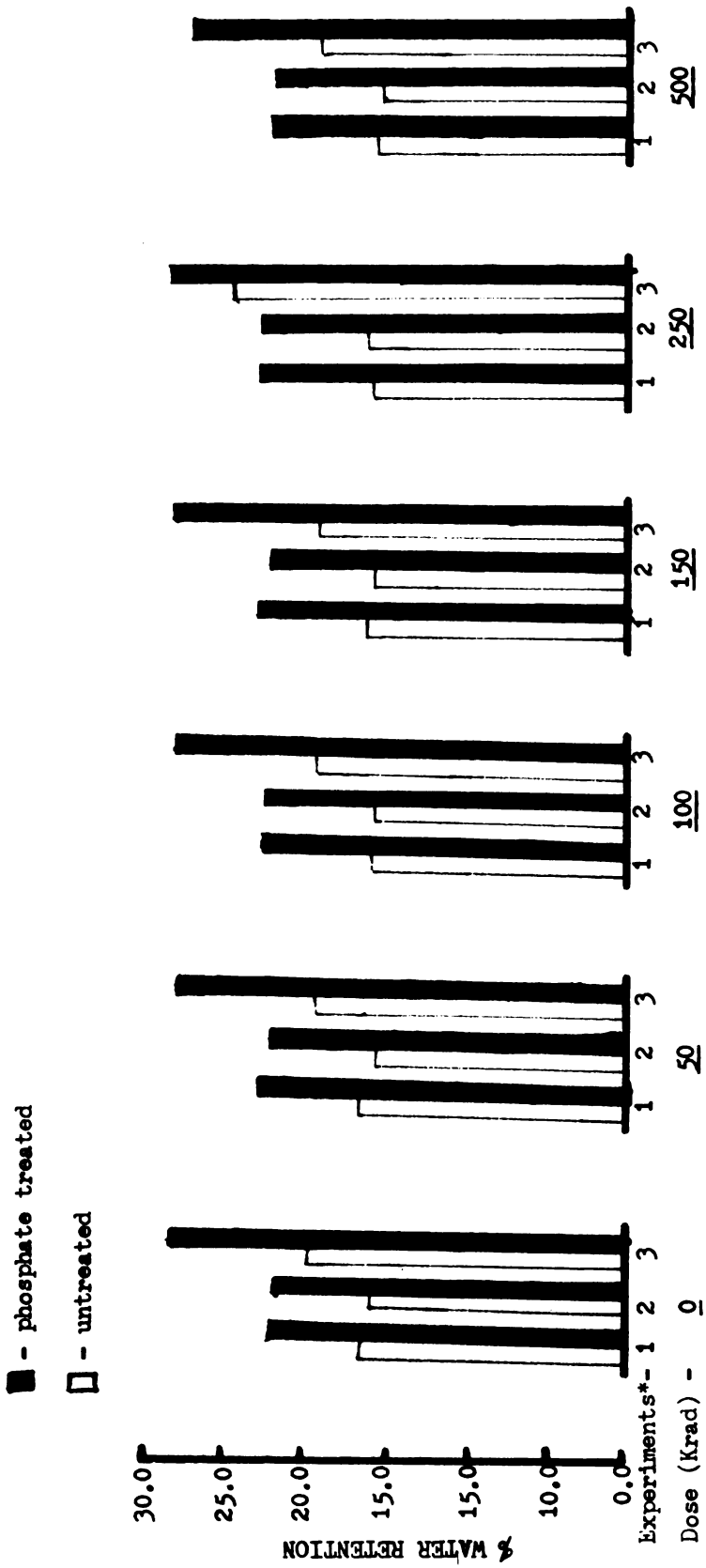

Figure 6.--Percentage water retention of beef slices that were vacuum packed, irradiated at various pasteurizing doses of gamma radiation and then stored for 21 days at 38°F. The percentage water retention of beef slices that were treated with sodium tripolyphosphate solution prior to irradiation is compared with the water retention of beef slices that were not treated.

* - Identical experiments performed on different days with different pieces of meat.

In Figure 7, the percentage water retention during storage of beef slices treated with sodium tripolyphosphate solution prior to irradiation at 100 Krad is compared with the percentage water retention of beef that was not treated. The percentage water retention of unirradiated beef samples with and without phosphate treatment is also compared. It is shown that phosphate treated samples have relatively high percentage water retention compared with the untreated ones. No significant changes in water retention due to irradiation at 100 Krad was observed. There was, however, a slight increase in water retention in all cases during storage for 21 days at 38°F.

Effect of Phosphate Pretreatment, Vacuum Packaging and Radiation on Lipid Oxidation in Fresh Beef

The object of this part of the study was to determine the degree of lipid oxidation that has occurred on beef samples after 21 days at 38°F and the effect of phosphate treatment, vacuum packaging and irradiation on lipid oxidation. Also of interest was the effect of exposing the meat to the atmosphere after 21 days in vacuum on lipid oxidation.

Beef slices (1 to 2 cm thick) from ribeye muscle of "Commercial" grade rounds were dipped in 10.0% aqueous solution of sodium tripolyphosphate and vacuum packed in oxygen impermeable film (IKD Super All-Vak #13). The samples were irradiated at 0, 50, 100, 150, 250 and 500

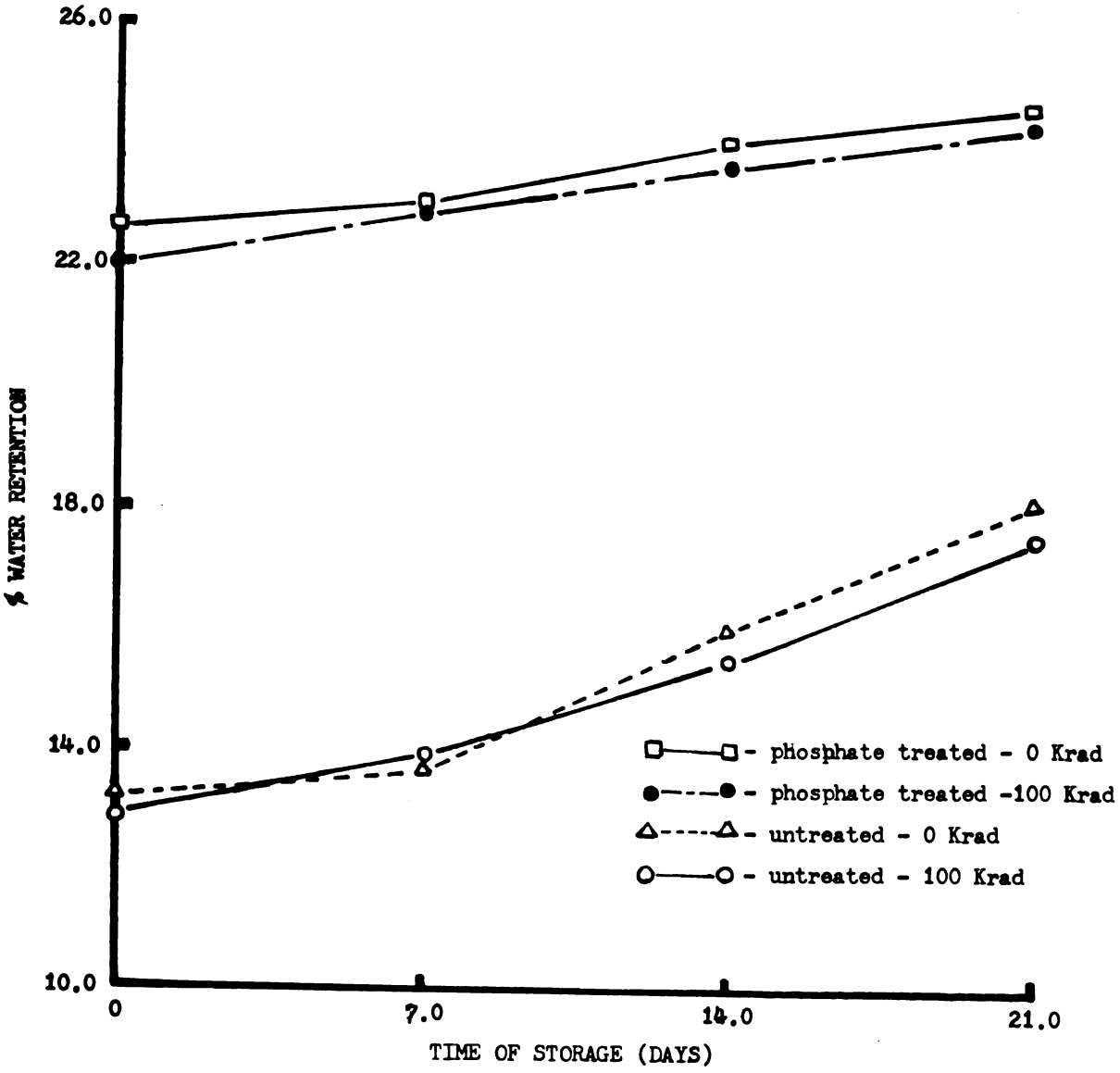

Figure 7.--Water retention during storage at 38°F of irradiated fresh beef pretreated with sodium tripolyphosphate.

Krad and stored at 38°F. Samples which did not receive phosphate pretreatment were exposed to the same dosages. At the end of 21 days, the vacuum of each pouch was broken. Representative samples were taken from the individual slices immediately upon opening and analyzed for TBA number. The remaining portion of the slice was rewrapped with oxygen permeable film and exposed to the atmosphere at 38°F. Daily measurements of lipid oxidation (TBA number) were conducted during this time. Results of these experiments are shown in Table 2.

It can be observed (Table 2) that vacuum packaging retarded lipid oxidation up to 21 days of storage at 38°F. This is evidenced by the relatively low TBA numbers obtained at the end of 21 days. Upon exposure to the atmosphere, however, TBA numbers began to increase with storage time up to three days. Differences in TBA numbers among the phosphate treated samples and untreated ones were less pronounced. No large differences in TBA numbers were observed between irradiated and unirradiated samples. In general, there appeared to be more variability in TBA numbers among meat samples used in various experiments.

In a separate set of experiments, the progression of lipid oxidation changes was followed during storage under vacuum and during the subsequent exposure to the atmosphere. Phosphate treated and untreated beef slices were vacuum packed and irradiated at 0 and 100 Krad. After

TABLE 2.--Effect of phosphate pretreatment, vacuum packaging, radiation and subsequent exposure to the atmosphere on lipid oxidation in fresh beef during storage at 38°F.

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

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

**O--untreated; P--phosphate treated.

[a]Average of two replicates

[b]Number of days exposed to the atmosphere.

irradiation they were stored at 38°F. At the end of 0, 7, 14 and 21 days, the samples were analyzed for TBA number. On the twenty-first day the samples were rewrapped with oxygen permeable film and stored for three more days at 38°F. Daily TBA number determinations were made during this time. Figure 8 gives the results. It can be observed that in all cases there was a slight increase in TBA numbers during the first seven days under vacuum. This however decreased from the seventh to the twenty-first day. No large difference in the degree of lipid oxidation between phosphate treated and untreated samples, and between irradiated and unirradiated samples was observed. Upon exposure to the atmosphere, however, there was a slight increase in TBA numbers in all cases on the first day and continued to increase up to the third day.

Results in the Preparation of Standard Curve for Pigment Determination

In the setting up of the assumed linear curve between the limiting K/S ratios at 571 mμ/525 mμ for 0 and 100% metmyoglobin, samples were obtained from the various ribeye muscles used in color and pigment changes studies. The preparation of the samples to obtain 100% myoglobin, 100% oxymyoglobin and 100% metmyoglobin was performed on the same day the various storage studies were set up. A total of sixteen determinations was accumulated and the results were summarized in Table 3. From the data, the standard

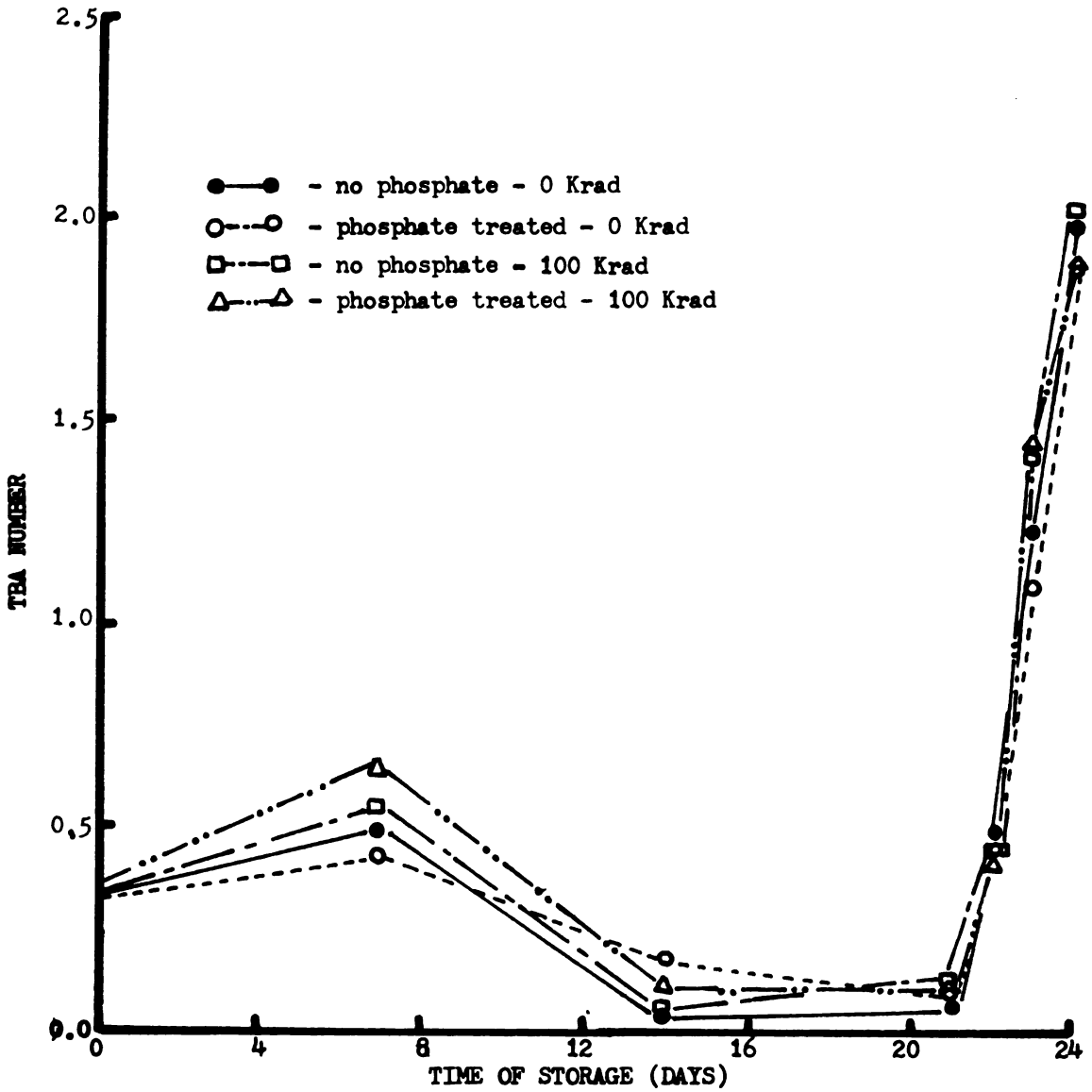

Figure 8.--Progression of lipid oxidation changes in irradiated fresh beef during storage in vacuum for 21 days followed by exposure to the atmosphere for three days at 38°F.

TABLE 3.--Ratios of K/S at $\frac{571\ m\mu}{525\ m\mu}$ for samples representing 100% myoglobin, 100% oxymyoglobin and 100% metmyoglobin.

Pigment	No. of Samples	$\frac{K/S \text{ at } 571\ m\mu}{K/S \text{ at } 525\ m\mu}$	
		Range	Average
Myoglobin	16	1.22-1.44	1.32
Oxymyoglobin	16	1.15-1.39	1.33
Metmyoglobin	16	0.52-0.66	0.59

curve for determination of the relative proportion of metmyoglobin was constructed. It is shown in Figure 9.

Table 3 summarizes K/S ratio at 571 mμ/525 mμ calculated from the reflectance readings of 16 samples. The average K/S ratio at 571 mμ/525 mμ are 1.32 for myoglobin and 0.59 for metmyoglobin. Stewart et al. (1965) reported K/S ratio at $\frac{572\ m\mu}{525\ m\mu}$ of 1.4 for myoglobin and 0.56 for metmyoglobin. The slight discrepancy in the values may be attributed to the fact that they used ground samples while in the present studies solid cut samples were used.

In using the K/S ratios at $\frac{571\ m\mu}{525\ m\mu}$, a linear relation is assumed between the ratios and the percentage metmyoglobin in the meat samples (Figure 9).

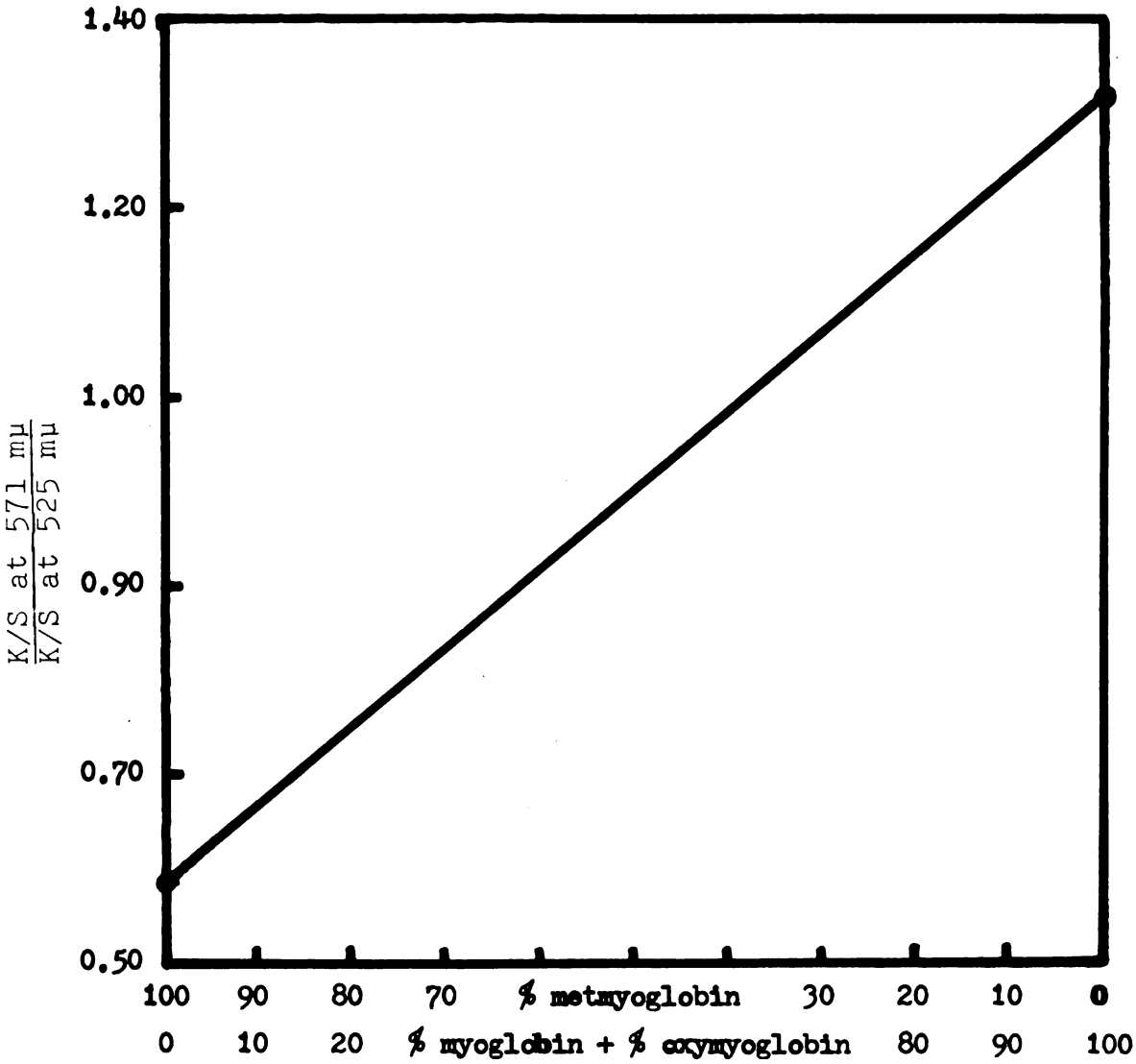

Figure 9.--Assumed linear relation between ratios of K/S at $\frac{571\ m\mu}{525\ m\mu}$ and the percentage metmyoglobin.

Effect of Phosphate Pretreatment, Vacuum Packaging, Radiation and Subsequent Exposure to the Atmosphere on Color and Metmyoglobin Formation in Fresh Beef

The purpose of this part of the work was to determine the combined effect of phosphate pretreatment, vacuum packaging and irradiation on the color of the fresh beef slices after 21 days storage at 38°F. Also of interest was the response with respect to the regeneration of red color at the surface of the meat during exposure to the atmosphere after storage in vacuum.

A separate set of experiments was conducted in this particular study. In order to reduce the variability of pigment constituents between muscles, samples were obtained solely from ribeye muscle of "Commercial" grade beef. The meat was trimmed of adipose tissue and cut into 1 to 2 cm thick slices. The slices were then dipped in sodium tripolyphosphate solution, drained and vacuum packed in oxygen-impermeable pouches. They were then irradiated at 0, 100, 200, 300, 400 and 500 Krad. Samples not treated with sodium tripolyphosphate were exposed to the same dosages. After irradiation the samples were stored at 38°F.

At the end of 21 days, the vacuum of each pouch was broken and the samples were prepared for reflectance measurements. Samples were cut into pieces having 1 3/4 x 1 1/2 inches dimensions, placed in plastic ice

cube holders and wrapped with oxygen permeable-film (Resinite RMF-61). The preparation of the samples and reflectance measurements were done quickly making exposure to the atmosphere as short as possible. Visual observations on the color of the samples were also made. The samples with oxygen-permeable film wrap were further stored at 38°F. After 24 hours, reflectance measurements were again made.

Experimental data presented in Table 4 indicate that there is a considerable difference in color between phosphate treated samples and untreated samples. The visual observation showed that in general, phosphate treated samples appeared purple in color while the untreated ones were brown after 21 days in vacuum. Reflectance measurements showed that reduced myoglobin predominates in phosphate treated samples while considerable amounts of brown pigment metmyoglobin are present in the untreated samples. Pasteurizing doses from 50 to 300 Krad did not show any detectable effect on metmyoglobin formation. At 400 to 500 Krad, there was a slight discoloration, with the phosphate treated samples appearing purplish brown while the untreated ones appeared brown.

Exposure of the meat to the atmosphere for a day at 38°F, shifted the color of phosphate treated samples from purple to red. Samples without phosphate pretreatment on the other hand remained brown. In all cases, there was an

TABLE 4.--Effect of phosphate pretreatment, vacuum packaging, radiation and subsequent exposure to the atmosphere on color and metmyoglobin development on fresh beef during storage at 38°F.

Dose (Krad)	Phosphate Treatment ***	Storage time (days) 21* Color (Visual observation)	21* % Metmyoglobin[a]	22** Color (Visual observation)	22** % Metmyoglobin[a]
0	O	Brown	11.5	Brown	28.2
	P	Purple	0.0	Red	2.0
50	O	Brown	9.0	Brown	24.7
	P	Purple	0.0	Red	3.2
100	O	Brown	5.0	Brown	18.0
	P	Purple	0.0	Red	7.0
200	O	Brown	11.5	Brown	28.0
	P	Purple	0.0	Red	3.0
300	O	Brown	10.3	Brown	27.5
	P	Purple	0.0	Red	9.5
400	O	Brown	21.2	Brown	43.0
	P	Purplish brown	9.0	Reddish brown	11.5
500	O	Brown	47.0	Brown	66.0
	P	Purplish brown	10.0	Reddish brown	20.2

*No. of days in vacuum.

**21 days in vacuum plus one day exposure to the atmosphere.

***O--untreated; P--phosphate treated.

[a]average of two determinations.

increase of metmyoglobin during exposure to the atmosphere. Reflectance measurements after exposure showed that both unirradiated samples and those irradiated at 50, 100, 200 and 300 Krad with phosphate pretreatment have lower percentages of metmyoglobin than samples that received similar dosages but were not treated with phosphate. The pigments in the phosphate treated samples appeared to be in oxygenated form as evidenced by the predominant red coloration. Phosphate treated samples that were irradiated at 400 and 500 Krad appeared reddish brown. The amount of metmyoglobin, however, is less than those samples that were exposed to 400 and 500 Krad but did not receive phosphate treatment.

Studies on the progression of pigment changes during storage were performed at different times using a different set of meat samples. Samples from ribeye muscle with 1 3/4 x 1 1/2 x 3/4 inches dimensions were prepared. After dipping in 10.0% solution of sodium tripolyphosphate, the samples were placed in plastic ice cube holders, vacuum packed in oxygen-impermeable pouches and irradiated at 0 and 100 Krad. Samples without phosphate treatment were exposed to the same dosages. After irradiation, the samples were stored at 38°F. At the end of 0, 7, 14 and 21 days, reflectance measurements were conducted directly on the samples while still in the package. At the end of 21 days, the vacuum of each pouch was broken. The samples were

rewrapped with oxygen-permeable film and stored at 38°F for three more days. Daily reflectance measurements were conducted during this time.

The progression of metmyoglobin formation during storage in vacuum and during the subsequent exposure to the atmosphere of phosphate treated and untreated beef slices irradiated at 0 and 100 Krad is shown in Figure 10. It is apparent that the percentage metmyoglobin is lower in phosphate treated samples than in untreated ones throughout the storage period in vacuum and during exposure to the atmosphere. There was a rapid increase in metmyoglobin formation beginning between the seventh and fourteenth days up to the twenty-first day of storage in vacuum in the case of beef slices without phosphate pretreatment. In phosphate treated samples, a slight increase in metmyoglobin was observed beginning between the fourteenth and the twenty-first days. At the end of 21 days in vacuum, all samples that did not have phosphate treatment appeared brown while the phosphate treated ones retained their deep purple color. Upon subsequent exposure to the atmosphere, the predominant purple color of the phosphate treated samples reverted to red color. No such reversion was observed with the untreated ones. There was, however, in all cases an increase in metmyoglobin at the surface of the meat samples after three days exposure to the atmosphere. It is also apparent from Figure 10 that irradiation

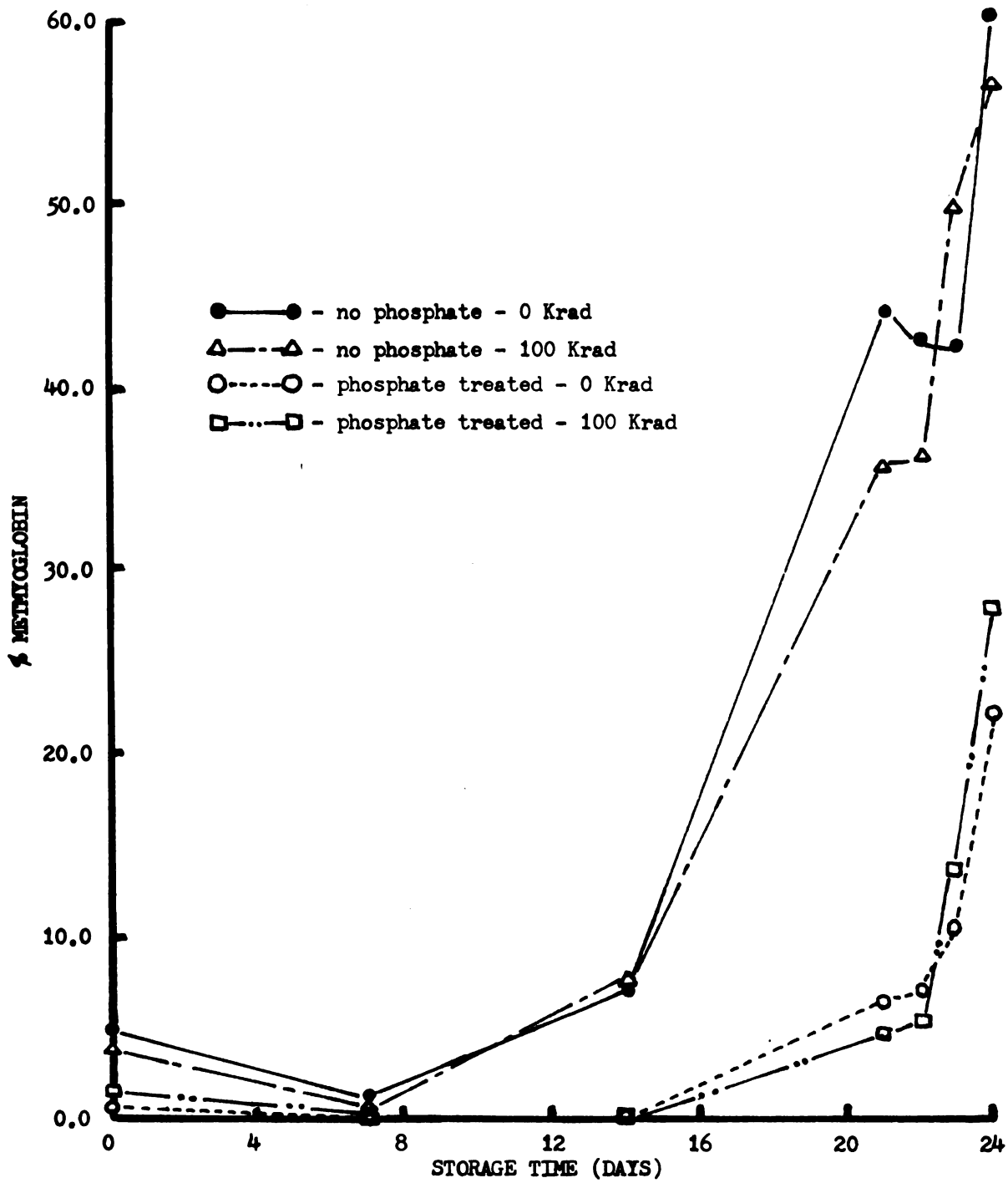

Figure 10.--Effect of phosphate pretreatment on metmyoglobin formation in irradiated and unirradiated fresh beef during storage for 21 days at 38°F followed by exposure to the atmosphere for three days.

at 100 Krad did not affect metmyoglobin formation in phosphate treated and untreated samples.

Organoleptic Evaluation (Color and Odor)

The combined effect of phosphate treatment, vacuum packaging, radiation and the subsequent exposure to the atmosphere on color and odor of the meat was tested. Beef slices from the ribeye muscles of commercial grade round were phosphate treated, drained, vacuum packed and irradiated at 0, 50, 100, 250 and 500 Krad. They were stored at 38°F. At the end of 20 days the vacuum of each pouch was broken. The samples were then rewrapped with oxygen-permeable film and exposed to the atmosphere for one more day at 38°F. Similar treatments were given to beef samples which did not receive phosphate pretreatment. Panel acceptability tests on color and odor were made after exposure to the atmosphere. Results are shown in Table 5.

The data of Table 5 show that the color of phosphate treated samples is superior to that of the untreated ones. Panel members distinctly preferred phosphate treated samples (colorwise) to the untreated ones. The mean color scores of phosphate treated samples ranged from fairly good to good (6.0 to 7.0), while those of the untreated ones ranged from fairly bad to marginal (4.0 to 5.0). There was no large difference in odor scores between phosphate treated and untreated samples. Unirradiated

samples and those that were irradiated at 500 Krad received lower ratings than those irradiated at 50, 100 and 200 Krad.

TABLE 5.--Mean color and odor scores of phosphate treated and untreated beef irradiated at various pasteurizing doses of gamma radiation and stored at 38°F for 20 days in vacuum followed by one day exposure to the atmosphere.

Experiment*	No. of Judges	Dose (Krad)	Phosphate Treated		Untreated	
			Mean Color Score**	Mean Odor Score**	Mean Color Score**	Mean Odor Score**
1	10	0	6.9	5.6	4.0	5.4
		50	6.5	7.4	4.5	7.0
		100	7.0	7.3	4.1	6.9
		250	6.8	6.8	4.1	6.2
		500	5.9	5.8	3.7	5.5
2	12	0	7.1	5.6	5.4	5.5
		100	7.2	7.0	5.3	7.2

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

**Scoring code:

Acceptable Range	Unacceptable Range
9 - excellent	4 - fairly bad
8 - very good	3 - bad
7 - good	2 - very bad
6 - fairly good	1 - poor
5 - marginal	

DISCUSSION

In vacuum packed beef slices which were radiation pasteurized and stored for 21 days at 38°F, a large amount of drip or exudate was observed in the packages. This drip formation in the package is undesirable from the standpoint of consumer acceptability. When the beef slices were dipped in sodium tripolyphosphate solution prior to vacuum packaging and irradiation, the amount of drip formed was reduced considerably. Also the water-holding capacity and appearance of the product were improved. This difference between the phosphate treated and the untreated beef samples clearly shows that phosphate pretreatment can compliment vacuum packaging and radiation pasteurization in the extension of the refrigerated shelf life (38°F) of fresh beef.

Irradiation at pasteurizing levels (50 to 500 Krad) did not have any effect on the formation of drip. There was, however, noticeable increase in drip formed due to the pressure exerted by the vacuum on meat samples. The amount of drip in untreated samples was markedly greater than in phosphate treated samples. Immediately after dipping in sodium tripolyphosphate solution, water-holding capacity of the beef samples increased. Phosphate pre-treatment also increased the pH of the meat. Irradiation

at 50 to 500 Krad did not produce any effect on the water-holding capacity. During storage, however, a slight increase in water-holding capacity was observed in both irradiated and unirradiated samples. This increase can be attributed to the phase of aging undergoing in meat during storage which tends to increase water holding capacity (Hamm, 1960). In all cases, phosphate treated samples maintained their high pH values (5.8-6.1) up to 21 days at 38°F. On the other hand, untreated samples showed lower pH values (5.2-5.4). The pH of the untreated samples after 21 days at 38°F is within the vicinity of the iso-electric pH (5.0-5.5) of the meat where there is a minimum water-holding capacity.

Drip control is obtained through the use of phosphate in an amount approximately 0.5% by weight. This amount is in accordance with the present U.S.D.A. regulations for those meats to which the addition of phosphate is permitted. At present these meats do not include fresh beef. Dipping does, however, result in marked but not serious increase in the slipperiness of the meat slices. The degree of slipperiness of the meat tends to dissipate during storage but still is perceptible after 21 days.

Vacuum packaging can effectively retard rancidity in radiation pasteurized meat. The low TBA numbers found during storage in vacuum suggests that exclusion of air during irradiation and storage can retard lipid oxidation.

The slight increase in TBA number during the first week of storage is presumed to be caused by the residual oxygen trapped in the muscle tissues. As the supply of oxygen began to decrease, however, the production of malonaldehyde as measured by the TBA test immediately dropped. The availability of oxygen is an important factor in the storage studies conducted with radiation pasteurized beef. Upon exposure to the atmosphere of the samples stored in vacuum, the degree of lipid oxidation increased.

Sodium tripolyphosphate has been shown to be an effective antioxidant in cooked meat and fish (Timms and Watts, 1958; Ramsey and Watts, 1963). Green and Watts (in press) reported that in raw meat, sodium tripolyphosphate did not show any antioxidant property. It is presumed that the phosphate groups are being hydrolyzed by the phosphatases in the muscle. The results presented in Table 2 and Figure 8 further illustrate the ineffectiveness of sodium tripolyphosphate as an antioxidant in fresh beef.

Irradiation in vacuum at 50 to 500 Krad did not cause any inhibition in lipid oxidation. The results are similar to those obtained by Greene and Watts (in press) in their studies on lipid oxidation on ground raw beef. All of the irradiated meats evaluated for odor in this study did not show any perceptible sign of rancidity after 20 days in vacuum and one day exposure to the atmosphere at 38°F.

Visual observations showed that in general, all meats pretreated with sodium tripolyphosphate had a superior color than the untreated ones. When meat slices were vacuum packed their color immediately changed to purple with the phosphate treated samples appearing darker than the untreated ones. It is presumed that the difference in the ultimate pH and water holding capacity caused such variability in the degree of darkness of the beef slices. A high pH has been shown to correspond to dark color (Hall et al., 1944; Bate-Smith, 1948; Janicki et al., 1967). The effect of pH may be based on the well known relation of pH on water holding capacity (Grau, 1953; Janicki and Walczak, 1954). In general, the darkening of meat color by added salts may be due to the increase of meat hydration. This may explain the influence of polyphosphate on meat color (Wismer-Pedersen, 1959d). Furthermore, since the darkness of meat color is related to the total energy reflected from the surface, any change in the physical and chemical properties of the meat would affect it (Janicki et al., 1967). As would be expected, darkness of meat color increases with the increase of water holding capacity and pH with the pigment remaining constant.

In general, radiation from 50 to 500 Krad did not show any immediate effect on the color of the vacuum packaged fresh beef. In some cases, however, brown coloration at the surface of the meat changed to a purplish

color during irradiation. This change is presumably due to the fact that irradiating in vacuum tends to generate reducing conditions in meat. Similar observation was also reported by Urbain et al. (1968). During storage, however, samples which have been exposed to 400 to 500 Krad showed some discoloration. Presumably, these dose levels (400 and 500 Krad) enhanced denaturation of the globin moiety of the heme proteins during storage.

Both in phosphate treated and in untreated samples which were irradiated in vacuum, the reduced form of myoglobin was the predominant pigment during the initial storage period. During storage, however, the amount of metmyoglobin increased. The brown metmyoglobin discoloration in phosphate treated samples was produced more slowly as shown by reflectance measurement which was employed to measure the relative proportion of metmyoglobin at the surface of the meat. On the other hand, a rapid rate of brown discoloration was observed in samples which did not receive sodium tripolyphosphate pretreatment. This difference in the rate of metmyoglobin discoloration can well be explained in that where oxygen is absent as in vacuum packaging the surviving activity of the cytochrome enzymes (in particular succinic dehydrogenase) can reduce metmyoglobin already formed (Lawrie, 1966). This reducing activity, however, is influenced by pH of the meat. It has been shown that as the pH decreases, the rate of

metmyoglobin reduction decreases (Cutaia and Ordal, 1964). Evidence has also been shown that autoxidation of mammalian myoglobin increases as the pH decreases (Matsuura et al., 1962). The effect of sodium tripolyphosphate treatment on pH of the meat would be the logical explanation of the observed changes in metmyoglobin discoloration during storage in vacuum of phosphate treated samples. Although the pH increase due to phosphate treatment in this experiment only ranged from 0.4 to 0.5 pH unit, this could have a marked effect on enzymatic reductions of metmyoglobin.

It is apparent that lipid oxidation and pigment discoloration can be retarded in radiation pasteurized fresh beef by the combined use of vacuum packaging and phosphate treatment. The purple color that predominates in vacuum packaged fresh beef, however, is not the typical color the consumers associate with fresh meat. Red color has been associated by the consumers with good quality meat. In order to regenerate the red color at the surface of the meat samples, they were exposed to the atmosphere after storage in vacuum. All of the irradiated meats which did not have phosphate pretreatment showed no regeneration of red color upon exposure. On the other hand, phosphate treated samples appeared red indicating that exposure to the atmosphere resulted in the oxygenation of the reduced myoglobin (which is predominant at the surface) to

oxymyoglobin. Longer exposure, however, resulted in increase of brown discoloration.

In general, there was no large difference in odor scores between phosphate treated and untreated samples. It is evident, however, that unirradiated samples and those that were irradiated at 500 Krad received lower odor scores than those at 50, 100, and 250 Krad. At times, panel members appeared rather inconsistent as individuals in reporting their opinion on the same samples from one observation to another. But they unanimously agreed that unpleasant changes were occurring in the unirradiated samples and those that were exposed to 500 Krad. Spoiled odor due to an apparent microbial spoilage seemed to predominate in unirradiated samples while slight irradiation odor was perceptible in samples irradiated at 500 Krad. Panel members did not observe a distinct rancid odor in all cases.

SUMMARY AND CONCLUSIONS

The use of sodium tripolyphosphate in conjunction with radiation pasteurization of prepackaged fresh beef was studied. Dipping beef slices in sodium tripolyphosphate solution of appropriate concentration prior to irradiation is compatible with radiation pasteurization of vacuum packed fresh beef slices. The pretreatment can compliment radiation pasteurization in that during storage, drip or exudate formation is minimized and the water holding capacity of beef is improved. Drip control is obtained through the use of sodium tripolyphosphate in an amount approximately 0.5% by weight.

The combined effect of phosphate pretreatment, vacuum packaging and radiation on lipid oxidation was also studied. From the TBA test data and odor evaluation, it was concluded that exclusion of air during irradiation and storage retarded lipid oxidation or rancid odor formation up to 21 days at 38°F. Phosphate treatment and radiation (50 to 500 Krad) did not show any effect on lipid oxidation in meat packed in vacuum.

Reflectance measurements and visual observations indicated that phosphate pretreatment in combination with vacuum packaging inhibited brown pigment discoloration at the surface of radiation pasteurized fresh beef. The

amount of metmyoglobin in phosphate treated samples was lower than in those samples that did not receive phosphate treatment. Phosphate treated samples retained their purple color up to 21 days in vacuum at 38°F while the untreated samples appeared brown.

Exposure to the atmosphere after storage in vacuum resulted in the regeneration of red color at the surface of the beef samples which have been pretreated with sodium tripolyphosphate. There was, however, indication of increased lipid oxidation and metmyoglobin discoloration in both phosphate treated and untreated samples during storage in air.

The odor evaluation showed that in general, all meats which were not radiation pasteurized had an odor which indicates microbial spoilage. At high pasteurization levels of radiation (500 Krad) there was a distinct irradiation odor noted by the panel judges. Those that were irradiated at 50 to 250 Krad showed no signs of such odors. Panel judges preferred the color of phosphate treated samples to that of the untreated ones after the samples have been exposed to the atmosphere for one day following 20 days in vacuum at 38°F.

Based on the experimental findings reported, it can be concluded that the three agents, radiation, phosphate treatment and vacuum packaging used in combination can extend the refrigerated life of fresh beef up to 21 days.

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