Changes Associated with Irradiating Meat and Meat Extracts with Gamma Rays

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An attempt has been made to determine the nature of the chemical changes in myoglobin, the major red pigment in muscle, that occur during irradiation and storage of meat or myoglobin preparations made from meat. The effects of gamma-ray radiation from a cobalt-60 source on myoglobin in extracts of meat and in muscle tissue were studied. Work was done with dilute purified metmyoglobin solutions, dilute and more concentrated crude extracts of meat, and meat in oxygen-permeable and impermeable packaging material, and the addition of ascorbic acid and effects of irradiation on storage properties of meat were studied. The susceptibility of myoglobin extracts to radiation damage increased with increased purity of the extract. Irradiation of myoglobin in crude extracts of meat gave inconsistent results, including oxidation and reduction reactions and the formation of a green compound which absorbs light at 610 to 620 mμ. It was shown that the porphyrin of this green compound has been altered by irradiation. Myoglobin in meat packaged and stored in oxygen-impermeable material shows little change immediately after irradiation or storage. Packaging in oxygen-permeable material results in discoloration immediately after irradiation and further discoloration on storage. The addition of 0.05 and 0.5% ascorbic acid was ineffective as a protective agent during irradiation.

IONIZING RADIATION for the preservation of food has recently attracted considerable interest. Dunn *et al.* (*10*) have found that ionizing radiation can be used to kill bacterial spores, yeasts, molds, and vegetative bacteria. However, foods develop off-odors and flavors and discoloration when exposed to sterilizing dosages of ionizing radiations. Available information indicates that the major red pigment in meat (myoglobin) is altered by irradiation treatment. Huber *et al.* (*16*) reported discoloration of muscle meats when exposed to high intensity electrons in doses as low as 150,000 rep. Brownell (*5, 6*), Hannan (*14, 15*), and Proctor (*19, 20*) all report discoloration when meat is irradiated under certain conditions.

Work was initiated in this laboratory to study the reactions of myoglobin with and without irradiation. Studies on the preparation and purification of myoglobin and on the determination of myoglobin in beef and pork muscle have been reported by Ginger *et al.* (*13*), Ginger and Schweigert (*12*), and Lewis and Schweigert (*18*). Work done on the effects of irradiation with gamma rays from a cobalt-60 source on this pigment in water extracts and in muscle tissue is presented in this paper, while changes in other constituents of meat during irradiation have been reported

by Doty and Wachter (*9*) and Batzer and Doty (*3*). In order to characterize the nature of the chemical reactions attributable to irradiation treatment, the initial studies were conducted with purified metmyoglobin and water extracts of meat. The results obtained in these studies served as a guide to subsequent studies conducted with fresh beef cuts.

Irradiation of Water Extracts of Meat

The extracts used were purified metmyoglobin (approximately 0.39 mg. of myoglobin per ml.) prepared as described by Ginger and Schweigert (*12*) and crude water extracts of meat (approximately 0.39 and 1.7 mg. of myoglobin per ml.). The latter extracts were prepared as follows: A quantity of fresh beef sirloin butt purchased at a local market was ground, suspended in an equal amount of water, and allowed to stand in the refrigerator overnight. It was then centrifuged, and the supernatant containing the myoglobin was obtained by filtration, diluted in certain cases, and used for irradiation studies.

Samples in test tubes were exposed to gamma rays from a 561-curie cobalt-60 source which was maintained at +4° C. After irradiation, samples were examined for gross visible changes and spectral absorption curves were determined using the Beckman DU spectrophotometer.

When purified metmyoglobin solu-

tions at pH 7.0 were irradiated with from 48,500 to 1.55 × 10⁶ rep, a gray-green-brown precipitate and a clear, colorless supernatant were obtained, indicating the precipitation of myoglobin along with other proteins. When crude dilute extracts of meat at pH 7.0 were exposed to radiation dosages up to 291,000 rep, there was no apparent alteration of the myoglobin.

More concentrated crude extracts of meat (1.7 mg. of myoglobin per ml.), when exposed to radiation doses ranging from 388,000 to 2.3 × 10⁶ rep, gave results which were not consistent. Extracts which appeared to be identical before irradiation, became green, green-brown, brown, or purple, or remained red on irradiation. The red-brown color was due to a mixture of metmyoglobin and oxymyoglobin, and the purple color probably was due to reduced myoglobin which on exposure to air became oxygenated to oxymyoglobin.

The production of the green color was of great interest, and attempts to determine the conditions in meat necessary for its formation have thus far been unsuccessful. However, addition of 50% ammonium sulfate to the crude extracts (with removal of the precipitate formed) before irradiation resulted in the formation of the green pigment. Experiments have not as yet been conducted to determine whether the presence of ammonium sulfate is directly responsible for increased formation of the green compound

or indirectly by removal of compounds by precipitation that may influence the amount of the green substance formed.

To help characterize the pigment, the light absorption spectrum of an extract, containing 50% ammonium sulfate, which turned green on irradiation, was obtained immediately after irradiation. The spectrum was similar to that of metmyoglobin, except that the 635-mμ peak of metmyoglobin had shifted to 610 mμ and was much more intense (Figure 1). This absorption band accounts for the green color of the solution.
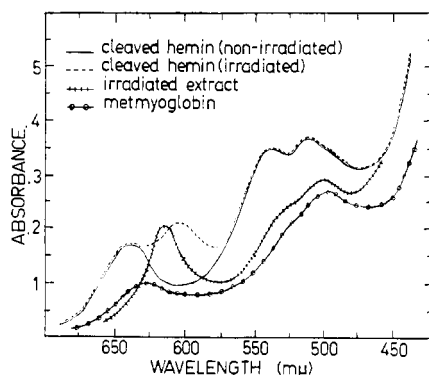

Figure 1. Spectral curves for green compound (irradiated extract), metmyoglobin, and hemin prepared from green compound and nonirradiated control

A portion of an extract which contained no ammonium sulfate but turned green on irradiation was treated with acid-acetone in order to cleave the iron porphyrin from the protein. For the cleavage, 2 ml. of the green solution was added to 20 ml. of acetone–hydrochloric acid (1 ml. of concentrated hydrochloric acid per 1000 ml. of acetone). The proteins precipitated and were removed by centrifugation. No color remained in the precipitate. The absorption spectrum of the cleaved green material in the acid-acetone is shown in Figure 1. This spectrum is probably a mixture of the green pigment with unmodified hemin. A nonirradiated control sample is identical with that obtained with crystalline hemin. The material from the green extract possessed a spectrum very similar to hemin, except for an additional peak at 605 mμ and a shoulder at 490 mμ. This is indicative of alteration in the porphyrin, which is in agreement with Barron (1), who states that during irradiation with sterilizing doses some of the porphyrin nucleus in foods may be destroyed.

Experiments showed that if an extract contains a high proportion of metmyoglobin, the latter may be converted during irradiation to a bright cherry red compound. However, if the extract contains a high proportion of oxymyoglobin, the formation of metmyoglobin and/or the green compound seems to be favored. Although the cherry red compound gave a spectral curve indicative of oxymyoglobin, there was some question as to its actual identity. In an effort to establish that it was oxymyoglobin, a control and irradiated extract were treated in a number of ways and the spectral curves were determined after each treatment.

Sodium hydrosulfite was added to both the control and the irradiated extract. This produced, in both cases, a purple colored solution which gave spectral absorption curves typical of reduced myoglobin, indicating the reduction of oxymyoglobin to myoglobin.

To each was added alkali to pH 11.8. This, in both cases resulted in typical alkaline metmyoglobin curves.

Potassium ferricyanide and sodium cyanide were added. The resultant spectral curves were typical of cyanmetmyoglobin for both samples.

Carbon monoxide was bubbled through both solutions and formation of carbon monoxide myoglobin occurred.

While these results do not conclusively prove that the red compound present after irradiation is actually oxymyoglobin, they lend support to this theory.

In general, it is believed that changes produced in foods by ionizing radiation are due to oxidation of the constituents of the food. In some of the experiments presented here, a change was observed which is apparently a reduction of metmyoglobin. It involved the reduction of iron from the ferric to the ferrous state. According to Barron (2), the reactions produced by ionizing radiation would favor the oxidation of free iron and of iron in cytochrome c, but the reduction of iron in bipyridine. It is, therefore, possible that the reduction of metmyoglobin to oxymyoglobin would take place, depending on the existent redox conditions.

Irradiation of Meat

The effect of irradiation on myoglobin in meat was studied in a series of 18 experiments involving 80 samples. The variables studied were the container in which the sample is irradiated (Visking casing, oxygen-permeable, and saran casing, oxygen-impermeable), irradiation dosage (48,500 to 2.3 × 10⁶ rep), and the presence of 0.5 and 0.05% ascorbic acid. In addition, the stability of myoglobin in irradiated and non-irradiated meat during storage was studied.

Comminuted choice grade sirloin beef butt was used except in two experiments involving 12 samples, in which whole meat chucks were used. Twenty- to 25-gram samples were packed tightly into the casings, excluding as much air as possible. After irradiation, gross observations were made and the samples were extracted overnight with water. The pH was then adjusted to 7.0, the

solution was filtered, and the spectral absorption curves were determined with the Beckman DU spectrophotometer. When it was found that the absorption curves did not show the differences seen on gross observation, the unextracted meat samples were used to determine the reflectance curves with the Beckman DU spectrophotometer, using the reflectance attachment supplied for the instrument.

Comparison of Visking and Saran Casing

Comminuted meat irradiated in saran casing showed less discoloration than that irradiated in Visking casing. When comminuted meat in saran casing was irradiated at 48,500 to 97,000 rep, it became slightly discolored, appearing slightly brown with red spots, or a red tan color. When the dosage was increased to 145,000 rep, the samples were a fairly good but faded red color. Irradiation with 1.55 × 10⁶ and 2.3 × 10⁶ rep resulted in samples which had a very good though slightly faded red color. In some cases where the sample was brownish before irradiation it was a very good red color after irradiation. This apparently is due to the conversion of metmyoglobin to myoglobin, which was then oxygenated to oxymyoglobin as was observed previously in the case of crude extracts.

Samples in Visking casings on the other hand became increasingly more discolored as irradiation dosage increased. Samples irradiated with 48,500 rep were either a fair red color or were red on the outside but brown in the center. When the dosage was increased to 97,000 rep, the reverse of this was sometimes noticed, while at a dosage of 145,500 rep and above the samples were brown outside with a red core, brown throughout, or a dull, purple-gray-brown color.

The discoloration of meat when irradiated in the presence of oxygen is in agreement with the observations reported by Hannan (14), Brownell, (5-7), and Brasch and Huber (4, 16).

Spectral absorption curves of comminuted and whole meat samples, in both Visking and saran casing, irradiated with 48,500, 97,000, and 145,500 rep showed a mixture of oxy- and metmyoglobin with an increase in metmyoglobin and a decrease in oxymyoglobin as the irradiation dosage became greater. This is contrary to the effect sometimes noted when high level dosage was used (1.55 × 10⁶ rep), in which metmyoglobin apparently was converted to oxymyoglobin. Comminuted samples in Visking casing irradiated with 291,000 rep gave spectral curves which indicated oxymyoglobin and another compound or two which gave absorption peaks at 500 mμ and shifted the 635- mμ metmyo-

globin peak to 615 to 620 mμ. When a comminuted meat sample in Visking casing was irradiated with 485,000 rep, the two oxymyoglobin peaks were observed as was the peak at 500 mμ, but there was a flattening of the curve in the 600 mμ area. Samples in both Visking and saran casing, which had been irradiated with from 1.55 $\times$ 10^6 to 2.3 $\times$ 10^6 rep, gave absorption curves which had the oxymyoglobin absorption peaks, had lost the peak at 500 mμ, and had a peak at 610 to 620 mμ. The peak at 610 to 620 mμ may be due to the green compound observed when extracts were irradiated.
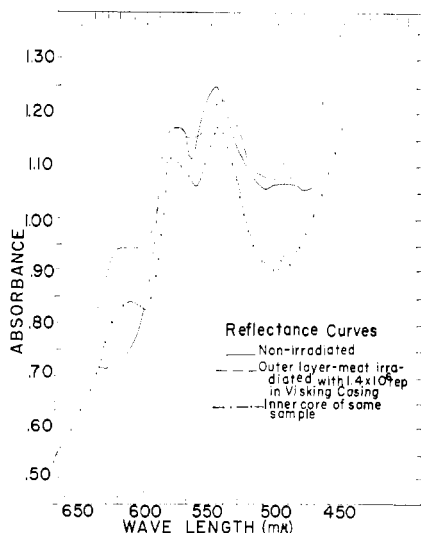

Figure 2. Reflectance curves of irradiated and nonirradiated comminuted meat

Reflectance curves were determined on a number of irradiated samples and representative curves are presented in Figure 2. The reflectance curves presented are a plot of the absorbance against wave length. Conventionally, reflectance curves are a plot of transmittance against wave length. The former method was chosen because it made the curves more meaningful for purposes of comparison with spectral absorption curves (17). The reflectance curves show a difference between the inside and outside of samples irradiated in Visking casing. The metmyoglobin on the inside of the sample seems to have been replaced by a compound which absorbs at 610 and 495 mμ. It may be concluded that some metmyoglobin is still present on the outside of the sample along with a compound absorbing light at 495 mμ and 610 to 620 mμ. This compound is probably the green pigment noted in the irradiated extracts and in the extracts of irradiated meat. The peaks at 545 and 580 mμ are indicative of the presence of oxymyoglobin.

Effect of Ascorbic Acid

In postulating a free-radical mechanism for the reactions taking place

during irradiation, the addition of a free radical acceptor such as ascorbic acid may be expected to reduce changes produced in the product being irradiated. Hannan (14) found that off-flavors in irradiated meat may be prevented to some extent by the addition of 0.5 to 1.0% sodium ascorbate. He points out that the mechanism of protection is not necessarily a direct competition for free radicals, since addition of the ascorbate after irradiation is almost as effective in preventing off-flavors as addition prior to irradiation. Proctor et al. (20, 21), who postulated a free radical mechanism, found that 0.5% ascorbic acid was effective in protecting the color of cured chopped meat products during irradiation with 2 $\times$ 10^6 rep. To test the effectiveness of ascorbic acid in protecting the color of fresh meat during irradiation, a series of experiments was done in which 0.05 or 0.5% solid ascorbic acid was thoroughly mixed with comminuted meat samples just prior to irradiation in either Visking or saran casing.

It was found that the ascorbic acid had a detrimental effect on the appearance of the meat. In general, irradiated samples in Visking casing, containing ascorbic acid, were as much, if not more, discolored than were those which were irradiated without ascorbic acid. Samples in saran casing without ascorbic acid were generally a good red color after irradiation. The addition of ascorbic acid did not improve the color and in some cases seemed to increase discoloration.

Representative spectral curves ob-

Figure 3. Spectral curves of extracts of irradiated and nonirradiated meat in presence and absence of 0.5% ascorbic acid

Compounds present in nonirradiated preparations are oxymyoglobin (absorption maxima at 545 and 580 mμ) and a small amount of metmyoglobin (absorption maxima at 500 and 635 mμ). Note also reduction in oxymyoglobin in irradiated samples and formation of the compound absorbing at 620 mμ when ascorbic acid was not used.
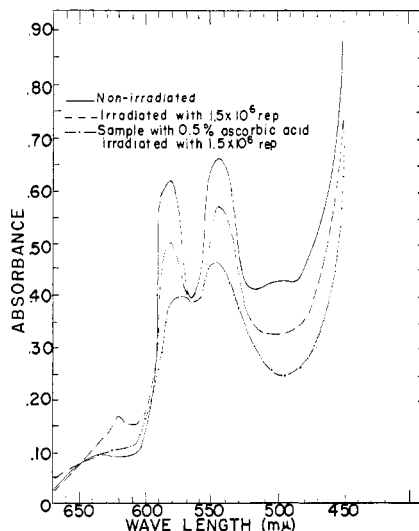
tained from extracts of irradiated meat with and without 0.5% ascorbic acid are presented in Figure 3. Samples in both Visking and saran gave the same curves, although gross appearance was different. The samples in Visking were far more discolored than those in saran. The curves obtained in the presence of 0.05% ascorbic acid were not too different from those obtained in the absence of ascorbic acid. The shoulder observed in the curve at 575 mμ when 0.5% ascorbic acid was added began to form after treatment with only 48,500 rep. The formation of this shoulder was not accompanied by a shift of peak from 635 mμ to between 620 and 610 mμ and the disappearance of the peak at 500 mμ. The curve is similar to that presented for the hydrogen peroxide metmyoglobin (8). In the presence of 0.05% ascorbic acid the shoulder did not form even after 2.3 $\times$ 10^6 rep. Instead, the peak at 500 mμ disappeared and the peak at 635 mμ shifted to between 620 and 610 mμ. When a sample was irradiated with 1.5 $\times$ 10^6 rep, this shift and disappearance seemed to be complete.

From these results it may be postulated that irradiation converts metmyoglobin in meat to a green compound which absorbs light at 620 to 610 mμ. The rate of this reaction may be increased in the presence of 0.05% ascorbic acid. In the presence of 0.5% ascorbic acid either a different reaction or a subsequent one occurs, which removes the compound that absorbs at 620 to 610 mμ as quickly as it is formed. Further studies should be carried out to ascertain what actually occurs.

Storage Experiments

The effect of irradiation on the stability of meat pigments during storage was also studied. Samples irradiated in Visking and in saran casing were studied. After irradiation with 145,000 or 1.55 $\times$ 10^6 rep the samples were left in the casings, wrapped loosely in waxed paper, and stored from 6 to 28 days in the meat drawer of the refrigerator at 1° to 4° C. Samples were withdrawn at intervals of 1 week, gross observations made, extracts obtained, and spectral curves determined.

Samples in Visking casing showed discoloration when irradiated, while those in saran casing did not. During storage, the samples in Visking casing, both irradiated and nonirradiated, became increasingly discolored. After 2 weeks' storage they had a spoiled meat or a yeasty odor, and were dried out and completely unacceptable. Samples, irradiated and nonirradiated, in saran casing retained a good red color throughout storage, although a slight amount of fading and dulling was noticed. The dullness generally disappeared when the sample was exposed to air. One set of

nonirradiated samples, in both Visking and saran casing, was stored for 76 days. At the end of this time the sample in Visking casing was brown, dried out, and completely unacceptable. The sample in saran casing was a somewhat light purple color, which changed to a good bright red color when exposed to air.

Spectral curves showed the samples in saran to contain mainly oxymyoglobin, while those in Visking contained mainly metmyoglobin. Presumably any reduced myoglobin obtained from samples stored in saran was converted to oxymyoglobin in the course of preparing the extracts.

The browning (formation of metmyoglobin and related compounds) of the irradiated meat in Visking casing during storage under these conditions is presumably due in part to dehydration of the samples. The shelf life of fresh beef irradiated with low dosages of gamma rays (60,000 rep) and stored in a moist chamber, however, was extended fivefold over controls held under identical conditions (Felton and Niven, 11).

Acknowledgment

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Literature Cited

(1) Barron, E. S. G., Proceedings of Sixth Research Conference, Council on Research, American Meat Institute, University of Chicago, March 25–26, 1954.
(2) Barron, E. S. G., Radiation Research, 1, 109 (1954).
(3) Batzer, O. F., and Doty, D. M., J. Agr. Food Chem., 3, 64 (1955).
(4) Brasch, A., and Huber, W., Science, 18, 536 (1948).
(5) Brownell, L. E., Eng. Research Inst., Univ. Mich., Utilization of Gross Fission Products, Progress Rept. 1 (August 1951).
(6) Ibid., 2, (January 1952).
(7) Ibid., 5 (September 1953).
(8) Dalziel, K., and O'Brien, J. R. P., Biochem. J., 56, 648 (1954).
(9) Doty, D. M., and Wachter, J. P., J. Agr. Food Chem., 3, 61 (1955).
(10) Dunn, C. G., Campbell, W. L., Fram, H., and Hutchins, A., J. Appl. Phys., 19, 605 (1948).
(11) Felton, E., and Niven, C. F., Jr., in preparation.
(12) Ginger, I. D., and Schweigert, B. S., J. Agr. Food Chem., 2, 1037 (1954).
(13) Ginger, I. D., Wilson, G. D., and Schweigert, B. S., Ibid., 2, 1037 (1954).
(14) Hannan, R. S., Food Sci. Abstr., 26, 121 (1954).
(15) Hannan, R. S., Research, 6, 376 (1953).
(16) Huber, W., Brasch, A., and Waly, A., Food Technol., 7, 109 (1953).
(17) Judd, D. B., "Color in Business, Science and Industry," John Wiley & Sons, New York, 1952.
(18) Lewis, U. J., and Schweigert, B. S., in preparation.
(19) Proctor, B. E., and Goldblith, S. A., Food Technol. 5, 376 (1951).
(20) Proctor, B. E., and Goldblith, S. A., Nucleonics, 10, 64 (1952).
(21) Proctor, B. E., Goldblith, S. A., Bates, C. J., and Hammerle, O. A., Food Technol., 6, 237 (1952).

FEED DIGESTIBILITY

Chemical Method for Measuring Relative Digestibility of Animal Protein Feedstuffs

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A simple enzymatic method is useful in determining the relative digestibility of meat and fish by-products used as feedstuffs. The indigestible residues may be studied microscopically for identification of most of their constituents, which consist predominantly of vegetable fiber, hoof, colloidal organic matter, hair, fuzz, and charred meat in various proportions. Indigestible residues in meat scrap range from 2 to 25%, seldom exceeding 10% in samples of good quality. Limited studies on fish meal show a high degree of digestibility unless the product has been overheated. Blood meal is highly digestible when properly processed, but may be much less digestible if overheated in the drying process. Reproducibility of the method is good, with less than 1% difference between duplicate samples.

FEED MANUFACTURERS throughout the country are becoming increasingly concerned over quality of ingredients going into manufactured feeds. It is common for feedstuffs to be bought on specification and for feed manufacturers to analyze them for moisture, protein, fat, fiber, and in many cases mineral elements and certain vitamins. Microscopic examination of feed ingredients is being used increasingly as a tool in improving quality of manufactured feeds.

However, the usual chemical analyses and vitamin assays do not tell the whole story of feedstuff quality and much effort is being expended by feed manufacturers to find other measures of quality control.

Meat scrap, meat and bone scrap, and tankage are by-products of the packing and rendering industries and their specifications have been concerned mainly with crude protein, fat, fiber, and phosphorus. Useless or contaminating ingredients such as hoof, hair, manure, and stomach contents have been covered by the stipulation that the maximum permissible quantities must not be greater than "might occur unavoidably in good factory practice." Such specifications do not mention digestibility of the protein, which is one of the determining factors in the nutritional value of the feedstuff. This is especially important in those meat scrap and tankage samples which contain