CHANGES IN MEAT PIGMENTS AS A RESULT OF RADAPPERTIZATION

M. KAREL*, A. R. KAMAREI* and E. WIERBICKI**

*Massachusetts Institute of Technology, Cambridge, Massachusetts, U.S.A.

**U.S. Army Natick Research and Development Command, Natick, Massachusetts, U.S.A.

INTRODUCTION

We found, in common with other investigators, that irradiation induces color changes in both cured and uncured meats. Knowledge about the mechanism of these changes and about effects of different process variables is needed, to establish the optimal conditions for radiation processing.

The steps in radiation sterilization are listed below:

(1) Formulation (stitch pumping of desired ingredients, mixing and diffusion).
(2) Enzyme inactivation (by heating in a smokehouse or in a water bath to an internal temperature of 73-75°C).
(3) Packaging (under vacuum in flexible pouches or cans).
(4) Freezing (-40 ± 10°C).
(5) Gamma or electron irradiation (26-44 kGy) at above temperature.
(6) Non-refrigerated storage.

In uncured meats, upon enzyme inactivation, a characteristic brown color appears. Upon packaging, freezing and irradiation a bright red color is formed, which is unstable and upon exposure to air and light turns to brown color again.

In cured meats, upon incorporation of curing ingredients, a bright-red color of nitrosyl myoglobin(NOMb) appears, which becomes the pink color of denatured NOMb (DNOMb) upon heating. Upon packaging, freezing and irradiation, an undesirable color fading is observed. Previous studies, however, have shown that small amounts of sodium nitrate may be used along with the reduced additions of nitrite ("mixed-cure") to prevent fading of the cured meat color (Wierbicki et al., 1977).

MATERIALS AND METHODS

Myoglobin Derivatives: Beef and pork oxymyoglobin (MbO_2) and metmyglobin (metMb) were extracted and isolated by the method of Hardman et al. (1966) and purified by the method of Awad et al. (1963). Nitrosyl myoglobin (NOMb) was prepared by bubbling pure nitric oxide through deaerated pure metmyoglobin solution in cold and dark, to achieve simultaneous reduction of heme iron and addition of NO. NOMb was also prepared by reaction with nitrite (25 ppm) in presence of ascorbate (550 ppm). Complete deoxygenation of the system is crucial for this preparation.

Myoglobin derivatives were irradiated under vacuum in 5-ml ampules closed by self-sealing rubber caps.

Meat Samples: Beef and pork semimembranosus muscles were used as sources of meat samples. For cured samples, curing pickle was prepared by addition of desired amount of nitrite or nitrate to bulk cure (solution of 0.3% sodium tripoly phosphate (TPP), 2.4% sodium chloride, and 550 ppm ascorbate) and then injected by stitch pumping. Meats were stuffed into regenerated cellulose casings and stored at 2°C for 48 hours. After enzyme inactivation, samples were cooled, sliced, packaged and frozen in blast freezer.

Irradiation Facilities: Samples were irradiated with gamma radiation from a Cobalt-60 source which has been described elsewhere (Karel, 1975).

Spectroscopy: Absorption spectra for myoglobin derivatives were obtained on a recording Beckman Spectrophotometer Model 26. Reflectance spectra were obtained using a General Electric Hardy Recording Spectrophotometer.

Experimental: Characteristic peaks of pigments in the range of 450-750 nm and in the Soret band from 375-450 nm were determined before and after irradiation.

Subjective evaluation (1 = extremely poor; 9 = excellent) of meat color was conducted at room temperature and under fluorescent light by a 15-member trained panel.

Objective evaluation was conducted on four (or five) replicates of each of the samples. Reflectance spectra were obtained after 0, 15 (or 20), and 90 min exposure to air and light at room temperature. For spectrophotometric assays, the most suitable and homogeneous parts of the meat samples were used. A piece of white paper board at the back of each sample assured uniformity of reflectance measurements.

Along with reflectance spectra, CIE tristimulus values (X,Y,Z) were obtained. These values were then converted to total color difference (ΔE) according to 1976 color difference L*,a*,b* equations (CIE, 1976). In each group, average value of controls has been assumed as the base line for measuring this color difference. The total difference between two colors given values of L*,a*,b* for each is calculated from:

$$\Delta E_{CIE}(L^*a^*b^*) = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Residual nitrite and nitrate were determined, using modified AOAC method (1975) before smoking, after smoking, and after smoking and irradiation.

RESULTS AND DISCUSSION

When evacuated solutions of MbO_2, metMb, and NOMb are irradiated with increasing doses of gamma radiation at different temperature, some shifts in characteristic peaks and a progressive decrease in the Soret band are observed (Fig. 1 and 2). At any dose of radiation, decrease of Soret band is much higher at 3°C than in systems at -30 ± 10°C. When the radiation temperature is lowered to -80°C, there is only minor additional protection against radiation.

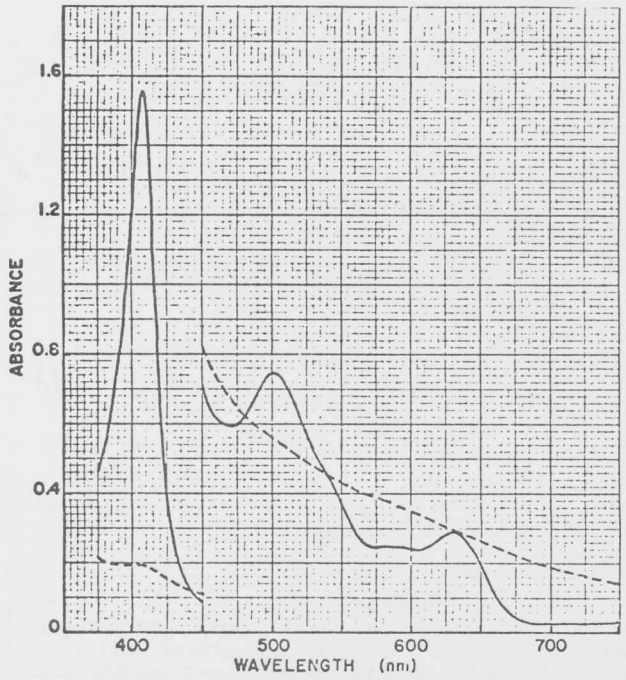

Fig. 1. Effect of irradiation (4 Mrad) at 3°C on absorption spectra of beef metMb solutions: Control(——),Irradiated(---)

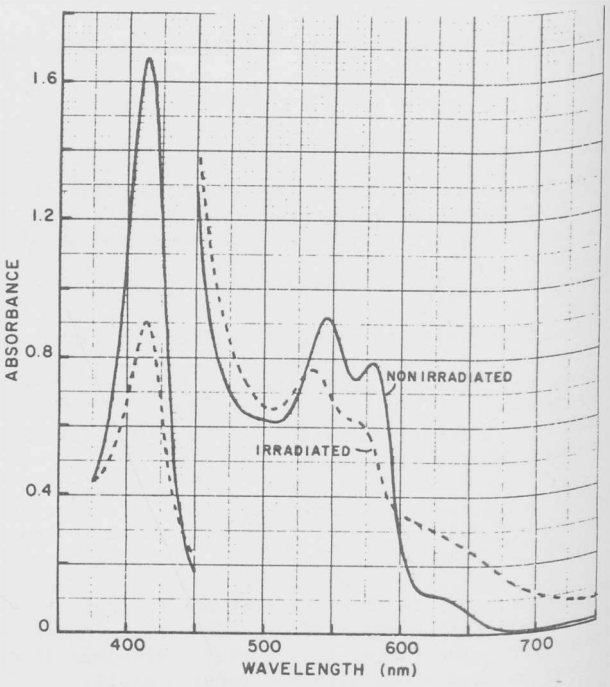

Fig. 2. Effect of irradiation (3 Mrad) at 3°C on absorption of porcine NOMb solutions: Control(——), Irradiation(---)

When nitrosylmyoglobin solutions synthesized with 25 ppm nitrite and 25 ppm nitrite plus 50 ppm nitrate, were irradiated, they did not show any significant differences in response to radiation.

As far as objective results for irradiated "uncured" meat is concerned, Figure 3 shows typical spectra representing the effect of radiation on the production of red pigment in meat (higher reflectance) and its gradual change to brown pigment (lower reflectance). The six spectra represent two sides of the irradiated beef sample after 0, 15 and 90 min of exposure to air. (radiation dose: 4 Mrad; radiation type: gamma rays; radiation temperature: 4 ± 2°C; exposure temperature: 5 ± 3°C).

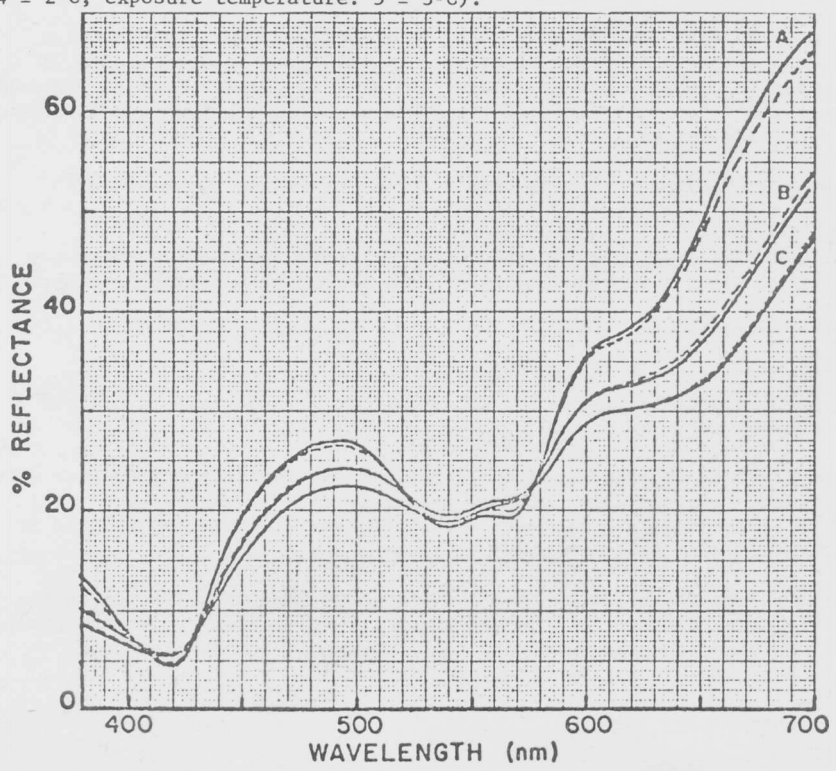

Fig. 3. Reflectance spectra of two sides of beef slices irradiated with gamma rays (4 Mrad) at 4°C, after exposure to air for 0(A), 15(B), and 90(C) min. spectrum C is also non-irradiated control.

It is obvious from these spectra that the reflectance intensity of an irradiated sample decreases with time. The spectra of non-irradiated beef do not change with time.

(1) Upon irradiation, the color of pre-cooked beef changes from brown to red, and, upon exposure to air, it returns to brown.
(2) The major change in reflectance from red to brown occurs during the first 15 min of exposure to air.
(3) The general pattern (peaks, maximum and minimum wavelengths) of the spectrum is preserved over time.
(4) In each group of samples, the lowest reflectance intensity (brown color after 90 min exposure to air) is similar to control spectra.

Comparing the formation of red pigment in irradiated metMb solutions with that in radiation-sterilized meat, which contains denatured metMb, shows that in both cases radiation is a dominant factor in producing the red color.

In heated meats, the dominant pigment is globin myohemichromogen. The heme iron in cooked meat is in the ferri state (Giddings, 1977), and, if reduced, will become reoxidized upon exposure to air (ferri iron cannot be oxygenated). We conclude that radiation reduces the brown pigment of cooked meats, and that, upon exposure to air, this reduced form of the compound oxidizes to the original brown compound that existed before irradiation. This suggested oxidation-reduction mechanism is shown in the following diagram(Kamarei et al., 1979):

$$\text{Mb, } MbO_2\text{, metMb} \xrightarrow[\text{enzyme inactivation}]{70\text{-}75^{\circ}} \underset{\text{(brown)}}{\text{globin myohemichromogen}}$$

(oxidation) ↑ Effect of air oxygen

(reduction) ↓ γ or electron radiation

globin myohemochromogen
(red)

Reduction of heme iron may be due to the presence of the hydrated electron, e^-_{aq}, which is formed during irradiation. If the suggested mechanism is correct, re-irradiation of radiation-sterilized beef samples should produce the red color again, and the red color so obtained should change to brown upon exposure to the oxygen in air. This experiment was performed, and the results were as predicted above.

Subjective studies of irradiated and non-irradiated "cured" meats with different levels of nitrite and nitrate (Fig. 4) show that the visual scores for those samples which contained nitrite or nitrate + nitrite, decrease, and for those without any curing agent or with nitrate, increase. Uncured samples become more acceptable upon irradiation due to development of bright red color. Panel members can not differentiate between this bright red radiation-induced color and the pink color of cured meats.

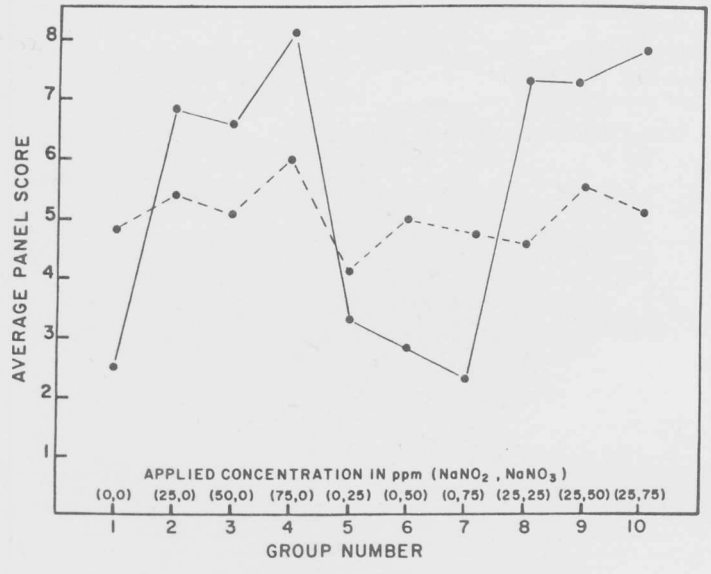

Fig. 4. Effect of 3.2 Mrad gamma radiation at -40°C on color rating of cured ham(control——, irradiated---).

Samples which contain only nitrate (groups 5,6,7) behave pre- and post-irradiation like uncured ham (group 1). Nitrate does not play a role in color development pre- and post-irradiation. Nitrate-containing samples, therefore, should be considered uncured and expected to behave as such, unless there is potential for reduction of nitrate to nitrite. Samples which contain only nitrite (groups 2,3,4) or nitrite + nitrate (groups 8,9,10) behave similarly pre- and post-irradiation.

However, radiation alters the pink color of cured ham to an unknown brownish color and therefore downgrades the color acceptability of these samples (radiation-induced fading). The mechanism of this fading is not clear and deserves further investigations. Objective studies using reflectance spectrophotometry and ΔE values confirm the subjective results.

Results of studies on residual nitrite and nitrate show that when nitrite alone is used, most of it reacts with different meat components (Cassens et al., 1977). However, upon smoking, a part of residual nitrite is oxidized to nitrate which is further destroyed by radiation. When nitrate alone is used, there is not any important reduction to nitrite at any stage. However, upon application of nitrate in all groups, about 15 ppm is reversibly complexed. Upon smoking, this nitrate as well as indigenous nitrate of meat can be measured as free residual nitrate. These higher concentrations of nitrate are significantly destroyed by radiation. Finally when nitrite and nitrate are used together, the behavior of nitrite is the same with nitrite alone. Behavior of nitrate is interesting. We found higher concentration of nitrate even in pre-smoking stage in these groups. One explanation can be that in presence of nitrite, the complexing sites of meat components are saturated. This as well as partial oxidation of nitrite and also indigenous nitrate will result to higher concentration of nitrate at this stage.

REFERENCES

AOAC. 1975. "Official Methods of Analysis" Association of Official Analytical Chemists, W. Horwitz (ed.) Washington, D.C. pp. 417-426.

Awad, E., Cameron, B. and Kotite, L. 1963. Chromatographic separation of hemoglobin and myoglobin on Sephadex gel. Nature, 189:1201.

Cassens, R.G., Woolford, G., Lee, S.H. and Goutefongea, R. 1977. Fate of nitrite in meat. In Proceedings of the International Symposium on Nitrite in Meat Products. Tinbergen, B.J. and Krol, B.(eds) p. 95.

CIE (Commission International del'Eclairage). 1976. Official recommendation on uniform color space, color difference equations and metric color terms. Suppl. 2, CIE Publication No. 15, (E-1.2.1).

Giddings, G.G. 1977. The basis of color in muscle foods. J. Food Sci. 5:103.

Hardman, K.D., Eylar, E.H., Ray, D.K. and Banaszak, L. J. 1966. Isolation of sperm whale myoglobin by low temperature fractionation with ethanol and metallic ions. J. Biol. Chem. 241:432.

Kamarei, A.R., Karel, M. and Wierbicki, E. 1979. Spectral studies on the role of ionizing radiation in color changes on radappertized beef. J. Food Sci. 44:25.

Karel, M. 1975. Radiation preservation of foods. In "Principles of Food Science", Part 2: Physical Principles of Food Preservation, Ed. Fennema, O. p. 93. Marcel Dekker, New York.

Wierbicki, E., Heiligman, F., and Wasserman, A.E. 1977. Irradiation as a conceivable way of reducing nitrites and nitrates in cured meats. In "Proc. 2nd Int'l Symp. on Nitrite in Meat Products. Tinbergen, B.J. and Krol, B. (eds.) p. 75.