Consumers, Cooks and meat processors daily observe the practical effect of heat on the pigments of fresh or cured meats. The doneness of a steak or roast has traditionally been judged on the basis of the color of the ready-to-serve meat. In the heat processing of meat, a major heat process restriction is the red cured meat color from nitric oxide myoglobin must be formed and stabilized.

The work to be described here was carried out at the Purdue University Department of Biochemistry and represents part of the Ph.D. dissertation work of Dr. Sharon W. Machlik.

We were lead into studying the heat precipitation behavior of the meat pigments by Dr. Machlik's observation during studies on the effect of heat on shear values of beef that solid cores of meat required higher temperatures of heating to attain equivalent precipitation of the total heme pigments than did ground meat from the identical beef semitendinosus muscles. Machlik and Draudt (1963) had shown that optimum or minimum shear values occurred on heating beef semitendinosus muscle to a temperature sufficient to cause shrinkage or melting of the collagen, yet not high enough to get into the hardening reaction temperature zone. It was desired to obtain some indication on whether or not this optimum condition for low shear values and thus, maximum tenderness could be judged from the color of the cooked meat.

The substantial difference between the behavior of ground and un-ground beef indicated that color might not be a good criteria of doneness and required a study of the behavior of the different meat pigments. Fresh (uncured) meat normally can contain a mixture of red oxymyoglobin, brown metmyoglobin and purple reduced myoglobin. Many factors not studied in this work, including such factors such as packaging, storage history as well as biological factors determine the quantitative relationship of these three pigments in a particular meat sample.

In cured meat, at the start of industrial heat processing, normally the major pigments are metmyoglobin and nitric oxide myoglobin. Metmyoglobin is formed on the addition of sodium nitrite and usually predominates. The ratio of pigments is dependent on many biological and processing factors that will not be covered here. In direct gas fired smoke houses some carbon monoxide myoglobin could conceivably be present.
It was shown by Bernofsky, et. al., (1959) and by Davis (1961) that for a given heating time and temperature purified myoglobin is much more stable during heating than when it is in combination with other proteins. The myoglobin apparently co-precipitates with other proteins and part of the thermal denaturation behavior of the pigments in meat has to be attributed to the denaturation of the other proteins of meat.

In order to gain an understanding of what might be occurring in practice during the heating of both uncured and cured meats, the effect of heating time and temperature was studied in three experimental systems.

1) Solid 1/2 inch diameter by 2-inch long cores, and 10 gram ground beef samples from the same semitendinosus muscle were heated under different conditions in 15 x 160 mm glass test tubes.

2) Ground beef semitendinosus muscle samples which had been chemically treated and handled in such a manner as to convert all of the pigment to specific desired derivatives, including metmyoglobin, oxymyoglobin, nitric oxide myoglobin, reduced myoglobin and carbon monoxide myoglobin, were heated under different conditions in 15 x 160 mm glass test tubes.

3) Highly purified beef skeletal muscle myoglobin derivatives including reduced myoglobin, metmyoglobin, oxymyoglobin, nitric oxide myoglobin, and carbon monoxide myoglobin, were heated at different temperatures for 30 minutes in 15 x 160 mm glass test tubes.

METHOD

PART I. Percent of Total Myoglobin Pigments Precipitated in Solid and Ground Beef When Heated for Different Times and Temperatures

All starting materials used throughout this work were good or choice grade beef semitendinosus muscles stored up to 30 days at -15° C. before use. For cutting solid meat samples cores, frozen semitendinosus muscles were thawed 15 hours at +4° C. and 1/2 inch diameter x 2 inch long cores were cut. Approximately 1/2 of the muscle was ground through a 1/8-inch plate. Ten gram portions of ground meat as well as cores were placed in 15 x 160 mm test tubes.

Preliminary tests indicated that when placed in a water bath in the 40 to 90° C. range in 15 x 160 mm test tubes, the core and ground meat center temperature comes to within 1° C. of the bath temperature in seven minutes. The bath temperature was held to ±0.1° C. of the specified temperature in all work reported here.

In all cases heating tests were carried out at the same time with solid and ground beef from the same muscle sample. Four identically
treated test tube samples were immediately chilled in ice water after a specific heat treatment and were blended 2 minutes in a chilled Waring blender and analyzed for total soluble myoglobin pigments using the cyanomet method of Drabkin and Austin (1935-36). Total soluble myoglobin from the same unheated ground control beef was measured as a reference.

PART II. Percent Reduced Myoglobin, Carbon Monoxide Myoglobin, Nitric Oxide Myoglobin, Oxymyoglobin, and Metmyoglobin Precipitated in Ground Beef by Heating Different Times and Temperatures

Fifty pounds of fresh good or choice beef semitendinosus muscles were ground, mixed thoroughly and frozen in 2-pound lots at -15°C.

All ferrous derivatives were made by placing a known weight of the ground beef in a plastic glove bag along with all equipment and reagents needed to form the derivative. The glove bag was flushed several times with specific gases to be used to remove oxygen. The specific purified gases used were, N₂ for reduced myoglobin, CO for carbon monoxide, O₂ for oxymyoglobin, and N₂ for nitric oxide myoglobin. These gases were allowed to saturate the spread out meat sample for 30 minutes. 0.1% dithionite was added to reduce any metmyoglobin present. For preparation of the nitric oxide myoglobin 0.02% sodium nitrite was added.

Three samples of each were blended, centrifuged, filtered and transferred to a stoppered cuvette in the glove bag under anaerobic conditions and were analyzed spectrophotometrically to confirm the presence of the desired derivative.

Approximately 10 gram samples were transferred to pre-weighed test tubes in the glove box and were capped with serum sleeves.

Sealed samples were removed from the glove box and held under a very slight positive nitrogen pressure and heated for various times and temperatures in the range of 35 to 95°C.

PART III. Heat Precipitation of Purified Beef Skeletal Muscle Myoglobin Derivatives

Fifty pounds of choice grade beef semitendinosus muscle was extracted with 50 liters of cold water. The extract was brought to 85% saturation with ammonium sulfate and the precipitate was discarded. The myoglobin derivatives were precipitated by bringing to 100% saturation. This procedure was carried out 3 times. After final precipitation, the crude myoglobin was desalted with G-25 sephadex. The solution was further desalted by dialysis, shell frozen at -15°C, lyophilized and stored under nitrogen at -15°C.

Since the thermal precipitation of myoglobin is known to be a function of the denaturation of other proteins, it was considered extremely important to remove other protein impurities.
Preparative Chromatography of Myoglobin

The above crude metmyoglobin (Mb') was chromatographed as the cyanide derivative on a preparative scale routinely on CM-cellulose (0.75 meq/g) that had been equilibrated with 0.01 M phosphate buffer, pH 6.9, containing 0.001 M potassium cyanide (Quinn, et. al., 1964). Mb', 800 mg, dissolved in 50 ml of water was dialyzed for 18 hours at 4° C. against 50 volumes of buffer before it was applied to a column (5 x 12 cm) of CM-cellulose which had been equilibrated with the same buffer used for the dialysis of myoglobin. Visual and spectrophotometric analysis indicated that the cyanmyoglobin was resolved into two fractions, labeled F1 and F2 in order of their emergence from the column. Twelve ml fractions were collected and analyzed spectrophotometrically at 415 and 280 mμ or 525 and 280 mμ. The major portion of the F2 fraction had 525 mμ/280 mμ ratio in the range of 3.9 to 4.5. Fox (1961) reported a ratio of 4.24 for aged myoglobin crystals.

To prepare Mb+ from Mb+CN, the Mb+CN had to be dialyzed several days against 0.6 M phosphate buffer, pH 5.8, to dissociate the cyanide. The Mb+ was analyzed spectrophotometrically to assure that complete dissociation of cyanide had occurred.

The purified Mb+ was concentrated by means of Sephadex G-200 (Sephadex Technical Data Sheet No. 1, Pharmacia, New York). The Mb+ was placed in dialysis tubing and then in a beaker. The dialysis tubing was covered completely with G-200 Sephadex, which has a high osmotic potential. The Sephadex was regenerated by washing with acetone and then air dried.

The concentrated purified Mb+ (CM-cellulose, F2 fraction) was dialyzed against acetate buffer, pH 5.6, I/2 = 0.1 for 18 hrs. The concentration of Mb+ was determined using the cyanmet method of Drabkin and Austin (1935-36). The purity of the myoglobin was estimated from the iron or heme content (deDuve, 1948) and the nitrogen content (micro-Kjeldahl, AOAC, 1955).

All purified myoglobin derivatives, except Mb+ were prepared under anaerobic conditions in a modified 100 ml glass tonometer immersed in an ice water bath.

Reduced myoglobin was prepared by bubbling nitrogen through the purified Mb+ solution for 15 minutes and added sodium dithionite (0.05%) by means of a hypdermic needle through a serum capped stopcock. Nitrogen was then bubbled through the solution for an additional 10 minutes.

MbCO was prepared by bubbling carbon monoxide through the Mb+ solution for 10 minutes. After reduction with dithionite, the carbon monoxide was bubbled through the solution for an additional 3 minutes.

MbNO was prepared by adding 0.02% sodium nitrite and 0.05% dithionite to the Mb+ solution.

Since direct oxygenation of Mb is known to result in the formation of some Mb+, MbO2 was prepared by first forming MbCO and then displacing the carbon monoxide with moisture saturated oxygen.
The myoglobin derivatives were analyzed with a Cary Recording DU spectro-photometer at wavelengths from 650 to 400 mu.

The thermal precipitation studies of purified Mb, MbCO, MbHb, MbO₂, and Mb⁺ were conducted in acetate buffer, pH 5.6, I/2 = 0.1 in order to study the thermal precipitation behavior of the various myoglobin derivatives under conditions similar to those found in meat. Zaiser and Steinhardt (1954) observed that denaturation velocities of hemoglobin derivatives were not affected when acetate buffer was used.

The concentration of myoglobin was 0.37 x 10⁻⁴ M. The myoglobin used for the precipitation studies was the CM-cellulose fraction F₂. This fraction was selected for several reasons. From starch gel electrophoresis studies, it was estimated that 85-90% of the F₂ fraction may be attributed to one myoglobin component. A higher yield of the F₂ fraction than F₁ fraction could be obtained from a single chromatographic experiment. Spectrophotometric analysis indicated that the 525 m/280 m ratio was more constant for a greater portion of this fraction than for the F₁ fraction.

Four ml aliquots of the myoglobin derivatives were transferred anaerobically to 15 x 160 mm test tubes which had been flushed with nitrogen. The test tubes were capped with serum sleeves and heated in a steam heated water bath for 30 minutes in the temperature range of 40° to 95° C. under a slight positive pressure of nitrogen.

After the samples had been heated, they were immersed in an ice-water mixture for approximately 10 minutes. The samples were then centrifuged and the myoglobin content was determined on 3 ml aliquots of the supernatant using the cyanmet method of Drabkin and Austin (1935-36).

Results and Discussion

PART I. Heat Precipitation of Pigments in Solid and Ground Beef

The thermal precipitation patterns for solid and ground beef are given in Figure 1. Each point on the graphs represents averaged results of 12 test tube samples. Results from 40° C. and 50° C. and above 69° C. are now shown since differences in percent precipitation were relatively small in these ranges.

**Figure 1**

In all cases in the range of 60 to 67° C. (140 to 152.6° F.) more myoglobin is precipitated under a given set of time temperature conditions with ground beef than with solid beef. This shift is roughly equivalent to a difference of 3° C. (5.4° F.) in heating temperature for equivalent precipitation.

PART II. Heat Precipitation of Ground Beef with the Pigment Converted to Specific Derivatives.

The thermal denaturation behavior of the various fresh and cured meat pigments are given in Figure 2.
Figure 1. Per Cent Myoglobin Precipitated in Ground and Solid Frozen Beef Semitendinosus Samples (10 g) Heated at 61°, 64°, and 67° C for Periods of Time Up to 120 Minutes.
Figure 2. Per Cent Mb, MbCO, MbNO, MbO₂, and Mb⁺ Precipitated in Model Systems of Ground Beef Semitendinosus Muscle (10 g) Samples Heated for 30 Minutes in the Temperature Range of 35°C - 90°C.
In the ground beef system 50 percent of the metmyoglobin (Mb⁺) heated under a slight positive pressure of purified nitrogen was precipitated after 30 minutes of heating at 44°C; whereas, 50 percent precipitation of Mb or MbCO required 30 minutes of heating at 72°C. Fifty percent precipitation of MbO₂ occurred at 55°C after 30 minutes of heating indicating an increased stability of 17°C over Mb⁺. Nitric oxide myoglobin is the most heat labile pigment of all. It exhibited 21 percent precipitation when heated at 35°C (95°F.) for 30 minutes and 50 percent precipitation when heated at 42°C (107.6°F.) for 30 minutes.

Heating Mb⁺ under oxygen increased the stability by approximately 15° - 20°C. The behavior of metmyoglobin blanketed with O₂ was much more heat stable than metmyoglobin blanketed with N₂. We have not found a satisfactory explanation for the increase in heat stability when Mb⁺ is heated in a pure oxygen atmosphere.

Considering the temperature to achieve 50 percent precipitation in 30 minutes the difference between the most stable pigments in the ground beef system - Mb⁺ and COMb, and the least stable - NOMb, is about 30°C (54°F.).

The stability of oxymyoglobin is intermediate between that of reduced myoglobin and metmyoglobin.

In the thermal denaturation studies of purified horse myoglobin (Fronticelli, et. al., 1962) no difference in thermal stability was observed between the ferro derivatives (Mb and MbCO) but a considerable difference in stability (11°C.) was observed between the ferric derivative, Mb⁺ and the ferro derivatives, Mb and MbCO. A 20° - 25°C. difference in stability between the same derivatives was observed in that study.

PART III. Heat Precipitation of Purified Beef Myoglobin Derivatives

The thermal precipitation patterns for purified bovine myoglobin are given in Figure 3. Each value in Figure 3 represents an average of 9 samples.

Figure 3.

A comparison of the results of Figure 2 with those in Figure 3 show that the thermal stabilities of the various myoglobin derivatives are in the same order relative to one another, in the purified system as in the ground meat system. However, the temperatures at which the derivatives precipitate are much higher in the purified systems.

Somewhat smaller differences in stability were observed among the various purified myoglobin derivatives than in the ground meat system. A 13°C. (23.4°F.) difference in the stability was observed between least stable purified derivative nitric oxide myoglobin and the most stable purified derivatives, Mb and MbCO as compared to the 30°C. difference observed in meat. However, this value for the purified derivatives is approximately 4°C. higher than that found for purified horse myoglobin by (Fronticelli, et. al., 1962). An increase in Mb⁺ stability was observed on heating the purified Mb⁺ under oxygen. We do not have an explanation for the increased stability of Mb⁺ in oxygen.
Figure 3. Per Cent Precipitation of Purified Mb, MbCO, MbNO, MbC02, and Mb+ in Acetate Buffer, pH 5.61, Heated 30 Min in the Temperature Range of 50° - 95° - 95°C.
In this study, 50% of the purified bovine Mb+ precipitated at approximately 62° - 65° C. after 30 minutes of heating; whereas, purified horse myoglobin (Fronticelli, et. al., 1962) began to precipitate at 68° - 70° C. after 15 minutes of heating. MbCO and Mb derivatives of horse myoglobin began to precipitate at 84° C.; whereas, the bovine derivatives began precipitating at 80° C.

Hatridge (1912) observed that both Hb+ and HbNO coagulated at 40° C. The spectral curves for HbNO and alkaline Mb+ were identical at 50° C. although a visual change in color of the solution was not observed.

According to Seidler and Schweigert (1959) the major portion of Mb+, pH 5.5, will be denatured after being heated one hour at 70° C. Davies (1961) found that Mb+ heated at 70° C. was slightly more stable and that 40% was denatured at 70° C. in one hour using a purified system. In this work at pH 5.6, approximately 25% Mb+ was precipitated in 30 minutes at 70° C.

The wide difference in the heat precipitation of the various pigments that can occur in fresh meat suggests that color is likely to be a rather poor indicator of degree of cookery.

The results here have important implications in the heat processing of cured meat products. Since nitric oxide myoglobin is far more easily heat precipitated than other derivatives, the desirability of early conversion of all of the pigment to the nitric oxide derivative during the heat process is indicated. This suggests that it is desirable to achieve reduction of the metmyoglobin formed to the ferrous form as early in the process as possible. It is theorized that accomplishing this will form the most pigment and the color will be "fixed" by denaturation at a relatively low processing temperature.

BIBLIOGRAPHY


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H. N. DRAUDT: Paper In.

HARRY BERNHOLDT: Thank you Ned. Moving on quickly to our next paper, Dr. George Wellington of Cornell University will speak on "Collagenolytic Activity During Low-Temperature Long-Time Heating of Bovine Muscle."

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