Effect of Wave Length of Light on Discoloration of Cured Meats

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The effect of the wave length of light upon the change from nitrosomyoglobin to metmyoglobin was studied in purified solutions of myoglobin and in ground cured meat. The oxidation of the pigment was determined by measuring the change in either the transmittance or the reflection spectrum. Through the use of filters the samples were exposed to equal intensities but different wave lengths of light. The action of light in the reaction was to hasten the dissociation of the nitric oxide from the myoglobin, thus rendering the pigment more susceptible to oxidation. In a solution of nitrosomyoglobin the effective wave lengths, which would result in the dissociation, were those absorbed by nitrosomyoglobin. With cured meat the effective spectral distribution curve approximated the absorption curve calculated from the reflection spectrum.

THE STORAGE LIFE OF CURED MEATS is markedly decreased in the presence of light and oxygen. Although this would appear to be a photochemical oxidation, it has not yet been demonstrated that it follows the general laws of photochemical reactions. A major factor preventing this demonstration is the difficulty involved in finding a particular spectral distribution curve which will promote the reaction.

Hockman (*8*) found that intensity seems to have a greater effect than does the color of light and that more fading occurred when the meat was exposed to near ultraviolet and less occurred with yellow light. Ramsbottom and others (*11*) found no difference in the discoloration of cured meat when ultraviolet light was excluded. They stated that cured meats were not so severely discolored by ultraviolet light as by fluorescent light under the experimental conditions, but that the rate of discoloration was the same when cured meat was exposed under equal intensities of incandescent and fluorescent light. Kraft and Ayres (*9*) state that ultraviolet light is apparently less harmful than soft white fluorescent light. Allen (*1*) showed no effects of light which could be attributed to the color of light in the visible spectrum, but Urbain and Ramsbottom (*14*) were able to show that boiled ham wrapped in red cellophane gave better color retention than ham wrapped in transparent cellophane.

Taylor and Pracejus (*12*) state that fading in most colored materials is a product of the intensity of light and time of exposure.

This investigation was designed to show the role of light in the discoloration of cured meat and to determine which wave lengths are responsible. The meat pigment, myoglobin, after conversion to the nitroso derivative, was used in the initial studies to determine the wave lengths of light which are most effective in the discoloration.

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Experimental

Myoglobin solutions were prepared by modifications of isolation procedures (*4, 6, 7, 10*). Finely ground beef chuck was extracted with an equal volume of water. The dark red supernatant was heated rapidly to 60° C., and cooled immediately and the precipitated proteins were removed. Further precipitation of proteins occurred when one-fifth volume of basic lead acetate was added to the supernatant. The excess lead was removed with phosphates, care being exercised to maintain a pH as near 7 as possible. The myoglobin was precipitated and fractionated with ammonium sulfate. The hemoglobin was removed with 3*M* phosphate buffer.

The myoglobin was reduced with sodium hydrosulfite and sufficient sodium nitrite was added to convert all of the pigment to nitrosomyoglobin. The amount of conversion to nitrosomyoglobin was determined by measuring the absorbance with a Beckman spectrophotometer. The extinction coefficients were determined by the method of Drabkin and Austin (*5*). A 0.06 millimole per liter solution of the nitrosomyoglobin was buffered at pH 7.5 for use in these studies. The high pH was used to slow the rate of dissociation (*13*).

The cured meat used in this investigation was the lean portion of boiled cured ham. The meat was ground three times through a plate with 1/8-inch holes and pressed tightly into small metal cups, which readily fitted the reflection unit of a Model DU Beckman spectrophotometer.

The relative energy of the light source was determined by the method described by Bachar, Coleman, and Hopkins (*2*), which consists of comparing the radiation of fluorescent tubes with a standard incandescent source: using both tubes as the light source for the Beckman spectrophotometer and measuring the illumination at various wave lengths. The voltage of the standard bulb was adjusted so that it would deliver 11.1 lumens per watt. Barnes and Forsythe (*3*) gave a graph of the spectral radiant intensities of a tungsten lamp when it is adjusted to this voltage. The relative energy at each wave length was then determined by taking the ratio of the illumination, as indicated by the spectrophotometer, times the spectral radiant energy of the standard.

The light was filtered through the following solutions:

Water, control
Copper sulfate, saturated solution at 37° F.
Potassium dichromate, saturated solution at 37° F.
Nitrosomyoglobin, 0.06 millimole per liter buffered at pH 10

The transmittance of these filter solutions was determined with the Beckman spectrophotometer before and after each experiment and no change was detected. The depth of the filters was constant during measuring and use. A fresh nitrosomyoglobin filter was used for each experiment.

Previous studies in this laboratory in-

dicated that the ratio of absorbances at wave lengths of 550 to 500 mμ would give an accurate measure of the concentrations of nitrosomyoglobin and metmyoglobin in a mixture of these two components. The myoglobin was prevented from oxygenating by adding a small quantity of potassium ferricyanide. It was not possible to detect the spectrum of oxymyoglobin at any time during the storage. The potassium ferricyanide also helped in preventing a recombination of the nitric oxide with myoglobin.

In the studies with cured meat the ratio of reflection at 650 to 570 mμ was used in determining the amount of discoloration, because the most pronounced change takes place after light exposure at these wave lengths.

Results and Discussion

The first step in this investigation was to demonstrate the action of light in the oxidation of nitrosomyoglobin. Myoglobin solutions buffered at pH 6.2 were reduced at low oxygen tensions with a small quantity of ascorbic acid. Two moles of sodium nitrite were added for each mole of myoglobin and the solutions were held in the dark at reduced air pressure for 1 hour. At the end of this period the spectrum indicated that from 95 to 100% of the solution was composed of nitrosomyoglobin. These solutions were divided and exposed, in thin layers, to varying radiant intensities and the spectra after exposure were determined. Ascorbic acid is not readily oxidized by the oxygen of the air and will permit the formation of oxymyoglobin in its presence. The pigment should, therefore, change to oxymyoglobin if a dissociation of the nitrosomyoglobin occurred. The samples which were stored in the dark remained as nitrosomyoglobin. With an increase in the radiant intensity more of the pigment was converted to oxymyoglobin.

This type of experiment demonstrated that the action of light caused the dissociation of nitrosomyoglobin. The oxymyoglobin was then formed, as this compound is not dissociated by light. If the formation of oxymyoglobin is prevented and the pigment placed in contact with oxygen, it is oxidized immediately when not associated with nitric oxide. In this investigation the amount of oxidation that occurred was used as a measure of the nitrosomyoglobin dissociation. This was thought to be a fairly accurate measure, as the spectrum of reduced myoglobin could not be detected.

The first experiments in determining the spectral distribution curve that is effective in the dissociation of nitrosomyoglobin were conducted with nitrosomyoglobin solutions, because small differences in the ratio of oxidized to reduced pigment could be measured by transmittance rather than reflection, as would be required with meat.

The relative energy of the light sources and the transmittance of the filters were known. It was, therefore, possible to adjust the filters by regulating the distance between the sample and the light source, so that the total relative energy transmitted by all 4 samples was equal. Figure 1 shows the relative energy transmitted through the four types of filters. The areas under these curves are equal and represent about 20 foot-candles.

Ten milliliters of nitrosomyoglobin were pipetted into 50-ml. beakers and placed under these filters at a temperature of 40° F. After 24-hour storage the ratio of the absorbance at 550/500 was determined. The average transmittance ratio of the nitrosomyoglobin solutions for 12 trials under these filters is presented in Table I. The analysis of variance showed these differences to be significant, with $P < 0.01$. The difference needed between any mean for significance at the 5% level was 0.06 and at the 1% level 0.08.

Table I. Transmittance Ratio of Nitrosomyoglobin after 24-Hour Exposure

Filter	Average Ratio 550/500
Water	1.25
Nitrosomyoglobin	1.34
Copper sulfate	1.18
Potassium dichromate	1.31
Darkness (40° F.)	1.52
Darkness (70° F.)	0.68

The high ratio indicates a solution which contains more nitrosomyoglobin and less metmyoglobin; therefore, less dissociation and subsequent oxidation have taken place. These results show that the wave lengths of light between 400 and 550 mμ are providing most of the energy that can be absorbed and used in the reaction.

The nitrosomyoglobin filter seems to be absorbing more of the energy which is effective in the dissociation of nitrosomyoglobin than any of the other filters. Attempts were made to fit the absorption curve of nitrosomyoglobin to the dissociation which took place under the various filters. The difference between the ratio with dark storage at 40° F. and the ratio under the various filters would be the dissociation due to that type of light. Making use of the absorption curve of nitrosomyoglobin, it is possible to calculate the expected dissociation, assuming all of the absorbed energy to be effective. Water was used as the standard in determining the transmittance of

Figure 1. Relative energy transmitted

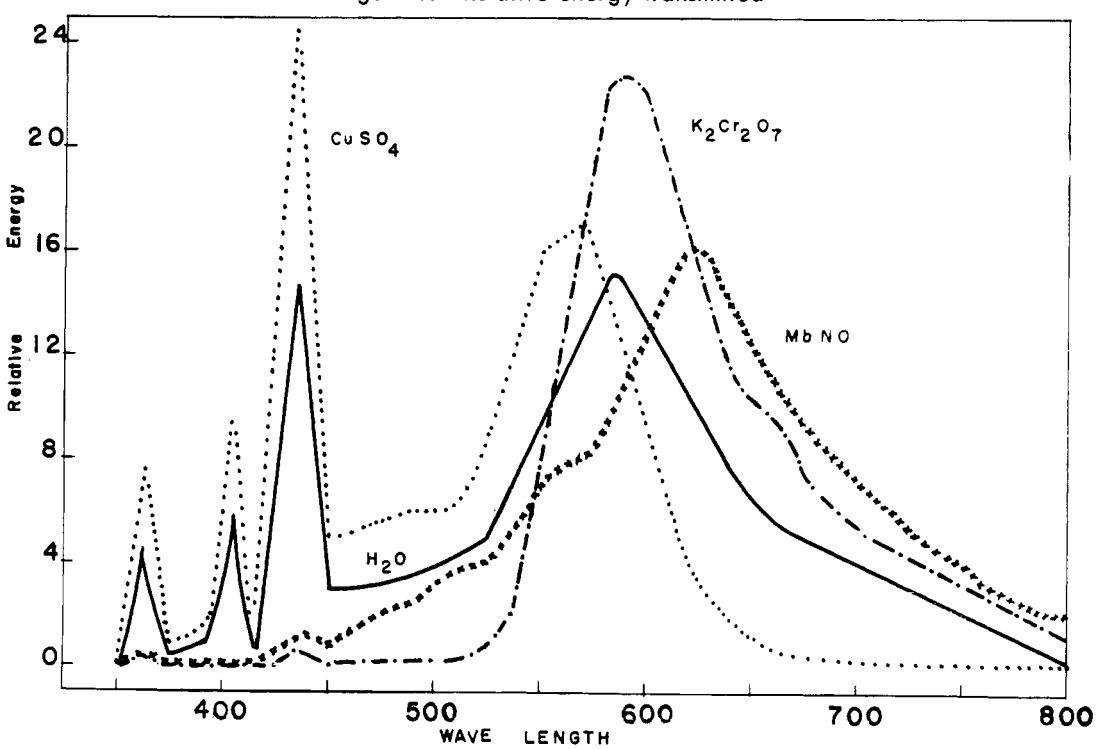

Table II. Dissociation of Nitrosomyoglobin

(Dissociation under water represents 100%)

Filter	Actual, %	Calculated, %
Water (standard)	100	100
Nitrosomyoglobin	67	65
Copper sulfate	126	127
Potassium dichromate	78	73

the filters and was, therefore, used as the standard in comparing the calculated and actual dissociation of the nitrosomyoglobin under the various filters. The actual and calculated per cent dissociation of nitrosomyoglobin are compared in Table II.

The effective wave lengths of the visible spectrum, in bringing about the photochemical oxidation of nitrosomyoglobin to metmyoglobin, would seem to be those absorbed by nitrosomyoglobin. It will be noted from Table I that heat as well as light energy will result in the dissociation of nitrosomyoglobin.

Table III. Reflection Ratio of Cured Ham after 24-Hour Exposure

Filter	Average Ratio 650/570
Water	1.69
Nitrosomyoglobin	1.82
Copper sulfate	1.68
Potassium dichromate	1.76
Darkness (40° F.)	2.16
Darkness (70° F.)	2.32

The nitrosomyoglobin in cured meat has undergone changes, such as denaturation, which could affect the reactions with light and oxygen. Samples of boiled ham, which were finely ground and thoroughly mixed, were exposed under these filters. The reflection ratio at wave lengths of 650 to 570 mμ was determined for 24 samples after 24-hour exposure under each filter and is presented in Table III.

The rate of dissociation was again compared with the absorption curve of a nitrosomyoglobin solution. The differences between the ratios do not correspond to those expected if nitrosomyoglobin, as it occurs in solution, is absorbing the energy. The absorption curve of the cured meat was determined from the reflection spectrum. It was also used in calculating the expected percentage dissociation (Table IV). The actual dissociation corresponded more closely to the absorption curve of the cured meat than to the absorption curve of undenatured nitrosomyoglobin in solution. This indicates that the absorption curve of nitrosomyoglobin is changed after heating the meat.

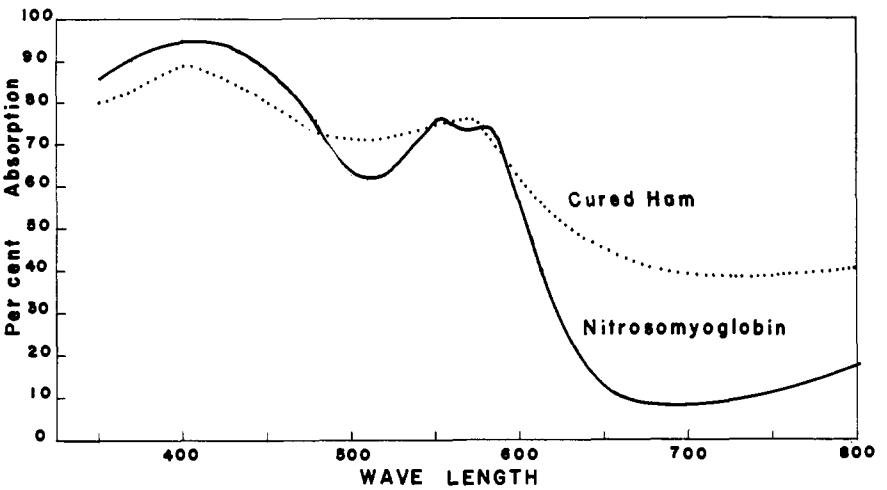

Figure 2. Average absorption

There are specific wave lengths of light which are absorbed to a greater extent by cured meat and bring about its discoloration. The effective spectral distribution curve is higher in the region between 400 and 550 mμ than at wave lengths above 550 mμ. The absorption curves of the nitrosomyoglobin and of the cured meat used in this experiment are presented in Figure 2. The spectral distribution curve which is responsible for the oxidation of cured meat seems to fall somewhere between the absorption curve of the entire meat sample and the curve for nitrosomyoglobin in solution. It is therefore possible to approximate the spectrum of the hemochromogen as it occurs in cured meat.

Table IV. Discoloration Compared to Water Filter

Filter	Actual, %	Calculated from Absorption Curve, %	
		Nitrosomyoglobin	Cured meat
Water (standard)	100	100	100
Nitrosomyoglobin	72	65	84
Copper sulfate	102	127	112
Potassium dichromate	85	73	88

A comparison of dark storage at 40° and 70° F. for nitrosomyoglobin (Table I) and cured meat (Table III) shows that the rate of dissociation of the cured meat pigment, unlike nitrosomyoglobin, is not increased by increase in the storage temperature.

Conclusions

In the visible spectrum the dissociation of a solution of nitrosomyoglobin is fostered by wave lengths of light that are absorbed by nitrosomyoglobin. With cured meat the effective spectral distribution curve is changed slightly, probably because of a change in the absorption curve of the denatured nitrosomyoglobin. The rate of dissociation of undenatured nitrosomyoglobin is increased by an increase in storage temperature; however, this effect disappears when the pigment is denatured.

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