Spectrophotometric Determination of Color Change in Cured Meat

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Determination of the extinction ratios of cured meat surfaces at 570/650 and at 540/500 mμ provides sufficient information to determine the extent of cured color development in freshly exposed cured meat surfaces, the amount of color fading, and the type of reaction responsible for color loss. Typical absorption curves are presented and extinction ratios for eight different types of cured meat are given.

THE RAPID INCREASE in sales of meats and meat products via the self-service counter has brought several problems, among which the discoloration of cured meats ranks high (*1, 3, 5*). The main problem is fading of the surface color of "ready-to-eat" meat products displayed in the lighted show counter. This color loss is caused by oxidation of the cured meat pigment from the ferrous to the ferric state, accelerated by light, which brings about dissociation of nitric oxide from the myoglobin, according to some workers (*2*). Walsh and Rose (*6*) recently proposed a different mechanism for the photo-oxidation of nitric oxide myoglobin, involving activation of the molecule by light.

The original bright pink color may be restored if such faded meats are subsequently stored in the dark, provided that excess nitrite and reducing groups (protein sulfhydryl or added ascorbic acid) are present. The light fading may be prevented altogether in the presence of sufficiently high concentrations of nitrite and a reducing agent (*8*).

Apart from this reversible fading, a second process may result in color loss. Peroxides, which in meats may be of bacterial origin or may be present as intermediates in unsaturated fat oxidation, destroy the porphyrin ring and the meat color fades to a greenish gray, distinctly different from the brownish metmyoglobin color (*1, 7*). This reaction is irreversible.

In the course of work with cured meats the need was felt for the objective measure of color fading. Ramsbottom, Goeser, and Shultz (*4*) and Kraft and Ayres (*3*) evaluated the surface color of bologna by measuring the ratio of light reflected at 650 mμ to that reflected at 570 mμ. They report a good correlation between these ratios and the visual color ratings. Fading of color paralleled the decrease in reflectance ratio. However, when dehydration occurred with the fading, the reflectance ratio increased and did not correlate with the visual score.

Watts and associates (*7*) made visual color comparisons using chemically treated cured meat slices as standards. They used a scale from 0 to 10, the standard for 0 being a slice of cured meat soaked in potassium ferricyanide and thoroughly rinsed with distilled water. This treatment gives the faded brownish color of the ferric denatured hemochromogen. The positive standard, designated 10, consisted of a freshly cut surface of the meat. To represent cleavage of the porphyrin ring, a slice of cured meat was treated with hydrogen peroxide, resulting in a complete loss of color. This standard was rated −10.

The present research was carried out to test the applicability of the spectrophotometric method as an objective measure of pigment fading in cured meats. Absorption curves have been plotted over the visible region for freshly exposed surfaces of a number of such meats, after oxidation of the pink ferrous cured meat pigment to the brown ferric compound by ferricyanide treatment, and after destruction of the porphyrin ring by hydrogen peroxide. By comparison of such curves, it was hoped to establish extinction ratios which would characterize the original cured meat pigment, its ferric oxidation product, and its final decomposition product. Such ratios should be useful in following objectively the degree of fading of a cured meat surface, in establishing the type of reaction responsible for fading, and in determining whether curing procedures have developed the maximum amount of cured meat pigment (nitric oxide hemochromogen) in various cured meats.

Materials and Methods

Eight different kinds of cured meat were obtained in bulk on the local market. Each was sliced in the laboratory and the slices were treated in various ways. Immediately afterwards, extinction and reflectance measurements were made between wave length 460 and 700 mμ, using a Beckman Model DU spectrophotometer with reflectance attachment. U.S.P. magnesium carbonate served as a standard. Extinction curves were plotted and visual scores assigned to all samples. The treatments included:

1. Freshly exposed surface (visual score 10).
2. Soaking for several hours in 0.1% potassium ferricyanide (visual score 0).
3. Soaking for 20 minutes in 0.05% hydrogen peroxide (visual score −10).
4. Exposing to incandescent light intensities of 15 or 200 foot-candles for various time intervals (protected from dehydration with a polyethylene wrap).

Results and Discussion

Absorption Curves of Freshly Exposed and Treated Cured Meat Surfaces. In Figures 1 and 2 are given representative extinction curves of a meat product with a high amount of pigment (salami) and with a low amount of pigment (Canadian bacon).

The freshly exposed surfaces of all tested meats showed a broad absorption peak at 570 mμ and very low absorption at wave lengths above 650 mμ.

The ferricyanide-treated samples had a much lower maximum absorption than the fresh meat, shifted to 540 mμ. At wave lengths above 640 mμ the absorption was higher than that of the fresh meat.

All light-faded meats showed absorption curves intermediate between those of the fresh samples and the ferricyanide-treated ones, approaching the latter as a limiting value. In all cases there was a definite absorption peak, shifting from 570 to 540 mμ with increased fading.

The absorption curves of the samples treated with hydrogen peroxide showed no peaks in the visible region, but gradually increasing absorption with decreasing wave lengths.

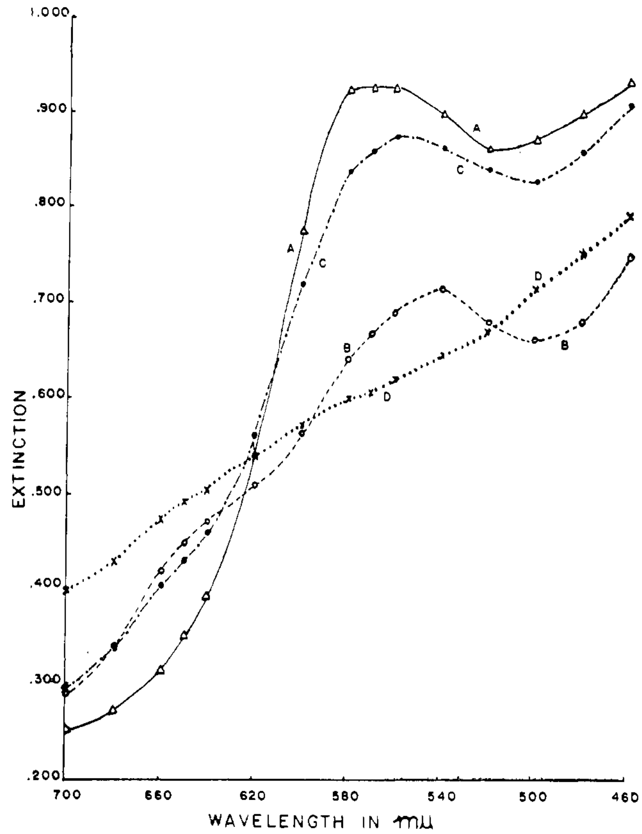

Figure 1. Extinction curves for salami

A. Freshly exposed surface B. Ferricyanide-treated surface
C. Light-faded surface D. Hydrogen peroxide–treated surface

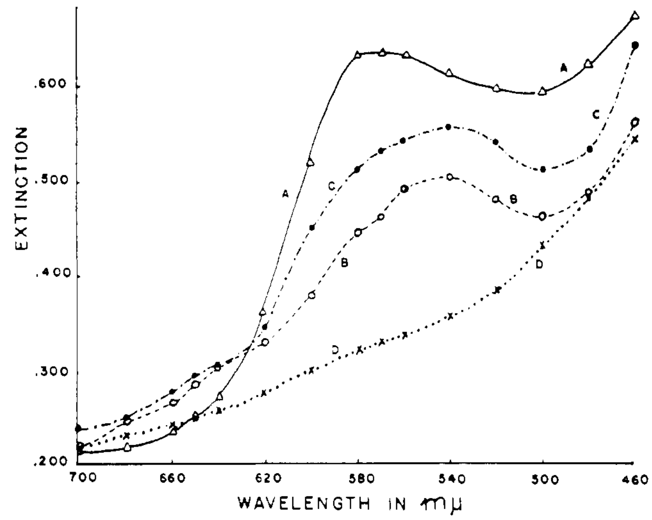

Figure 2. Extinction curves for Canadian bacon

A. Freshly exposed surface B. Ferricyanide-treated surface
C. Light-faded surface D. Hydrogen peroxide–treated surface

Characterization of Ferrous-Ferric Transformation. The wave length ratio which consistently shows the greatest difference between fresh and ferricyanide-treated meats is that at 570/650 mμ, in agreement with wave lengths selected by earlier workers to characterize faded meats (*4*). Extinction ratios of 570/650 mμ are much more meaningful in characterizing changes in the pigments than are the corresponding reflectance ratios of 650/570 mμ. Extinction ratios would be expected to remain the same for a given pigment, irrespective of the amount of pigment present at the surface examined (provided, of course, that the absorption was due entirely to the pigment), whereas reflectance ratios for the same pigment would vary widely with the percentage of light absorbed or reflected.

Thus in comparing the two samples of meat for which curves are presented, the extinction ratios are 2.59 and 2.42 for the salami and Canadian bacon, respectively, whereas the corresponding reflectance ratios are 3.76 and 2.41. It is not possible to make useful comparisons of reflectance ratios in different kinds of cured meats, or even in the same kind of meat, when the surfaces selected for examination differ in degree of pigmentation, amount of fat, etc.

The advantage of absorption over reflectance ratios is clearly shown in the case of light fading of meat accompanied by surface dehydration. This phenomenon is not at all rare in the lighted display counters as they are used in many stores. The darkening of the drying out surface results in a higher reflectance ratio than corresponds with the visual score. In the case of bologna, a light-faded dehydrated sample had a reflectance ratio of 3.10 and an extinction ratio of 1.67. The ratios for the fresh product were, respectively, 2.64 and 2.82. Thus, while the extinction ratio falls with pigment oxidation, corresponding to decreasing visual scores, reflectance ratios actually increase, which would indicate an improved product.

The ratios of the absorbancy at 570/650 mμ for all the freshly exposed meat surfaces examined varied considerably (Table I). The lowest value of 2.16, obtained on liver sausage, was far

Table I. Effect of Treatment on Extinction Ratios of Cured Meats

Treatment	*Type of Meat*	*Extinction Ratio* 570/650 mμ	540/500 mμ
Fresh surface	Canned comminuted ham	2.78	1.05
	Bologna	2.82	1.01
	Canadian bacon	2.42	1.02
	Salami	2.59	1.02
	Canned whole ham	2.61	1.05
	Canned comminuted pork	2.52	0.97
	Picnic ham	2.50	1.05
	Liver sausage	2.16 (2.51)	. . .
	Average	2.56 (2.59)	1.02
Treated with ferricyanide	Canned comminuted ham	1.61	1.04
	Bologna	1.55	1.02
	Canadian bacon	1.60	1.08
	Salami	1.49	1.07
	Canned whole ham	1.47	1.08
	Canned comminuted pork	1.62	1.03
	Picnic ham	1.63	1.03
	Liver sausage	1.47	0.98
	Average	1.56	1.04
Treated with hydrogen peroxide	Canned comminuted ham	1.40	0.93
	Bologna	1.39	0.78
	Canadian bacon	1.36	0.83
	Salami	1.24	0.90
	Canned whole ham	1.30	0.86
	Canned comminuted pork	1.37	0.96
	Picnic ham	1.36	0.96
	Liver sausage	1.25	0.88
	Average	1.33	0.89

below that for the other meats examined. To determine whether this low ratio represented incomplete development of the cured meat pigment, a sample of this sausage was dipped briefly in a solution containing 0.2% nitrite and 0.2% ascorbic acid (pH adjusted) and stored several days in the refrigerator. After this treatment, the sample was a pinker color than the original meat and the ratio had increased to 2.51. The average extinction ratio 570/650 mμ of all the meats examined was 2.56. With the improved color of liver sausage the average was 2.59 and without the liver sausage 2.61. It is possible that the ratios would be increased for some of the other meats by treatment with excess nitrite and reducing agents. Ratios below 2.4 on freshly exposed cured meat surfaces would indicate that full cured meat color had not been developed during curing. The diagnosis is confirmed if the ratio is increased by treating with nitrite and reducing agent.

Table I also gives the extinction ratios for the oxidized pigment. The average value of these ratios is 1.56. The difference between the two average extinction ratios is highly significant.

Absorption ratios of light-faded meats were all lower than those of the fresh product and approached those of the ferricyanide-treated samples with increased color loss. There was a close correlation between the extinction ratios and the visual score of the faded meats. The correlation coefficient of all samples tested was 0.90.

From the given data it is possible to calculate roughly the percentage of the original pigment in a light-faded product which is oxidized to the ferric form. A more accurate calculation can be made if the extinction ratios for the fully developed color and for the completely oxidized form of the meat under scrutiny are known. For example, the extinction ratio of fresh picnic ham is 2.50; after complete oxidation of the pigment the ratio is 1.63. After exposure to light of 200 foot-candles intensity for 1 hour the ratio is 1.92. This represents a pigment oxidation of 66%. Exposure to 15 foot-candles for 4 hours results in oxidation of 23% of the pigment (ratio 2.30).

Porphyrin Ring Destruction. The extinction ratios of the hydrogen peroxide–treated samples (Table I) are invariably lower than those of the reversibly oxidized samples of the same meat. The average value is 1.33, which differs significantly at the 1% level from the average ratio of the ferricyanide-treated samples.

Use of this ratio, however, to establish porphyrin ring destruction in cured meats is of questionable value, as iron oxidation and porphyrin ring destruction may go on simultaneously, so that at intermediate values a fall in this ratio could be ascribed to either type of chemical change in the heme pigment.

The ratio between the extinction at 540 and 500 mμ, however, provides the desired distinction between the two fading processes (Table I). The average value of the fresh and the ferricyanide-treated samples are close together. Formation of metmyoglobin does not result in a significant change in this ratio. When porphyrin ring destruction takes place, the value drops considerably. The average value for the extinction ratio at these wave lengths is 1.02 for the fresh surface, 1.04 for the oxidized pigment, and 0.89 for the hydrogen peroxide-treated samples. The difference between the averages of the fresh and the oxidized pigment on the one hand and the hydrogen peroxide -treated on the other hand is highly significant at the 1% level. From these data the amount of ring destruction can be determined in a similar way as for the ferrous-ferric oxidation.

Determination of the two extinction ratios given here makes it possible to establish the extent and the type of reaction taking place in a fading meat product.

Literature Cited

(1) Jensen, L. B., "Microbiology of Meats," 3rd ed., Garrard Press, Champaign, Ill., 1954.
(2) Kampschmidt, R. F., J. Agr. Food Chem. **3**, 510 (1955).
(3) Kraft, A. A., Ayres, J. C., *Food Technol.* **8**, 22 (1954).
(4) Ramsbottom, J. M., Goeser, P. A., Shultz, H. W., *Food Eng.* **23**, 120 (1951).
(5) Urbain, W. M., Ramsbottom, J. M., *Food Research* **13**, 432 (1948).
(6) Walsh, K. A., Rose, D., J. Agr. Food Chem. **4**, 352 (1956).
(7) Watts, B. M., *Advances in Food Research* **5**, 1–52 (1954).
(8) Watts, B. M., Erdman, A. M., Wentworth, J., J. Agr. Food Chem. **3**, 147 (1955).

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STARCH IDENTIFICATION

Microscopic Characteristics of Starches in the Identification of Ground Cereal Grains

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Observations made during various projects on starch and cereal grain characteristics are given to aid feed microscopists in the identification and quantitative estimation of cereal grain constituents in feeds. The microscopic appearance of ungelatinized and gelatinized starch granules can be used to distinguish the presence of most of the cereal grains. Microscopic structure of kernel parts can provide final distinction between grains that have similar starches, such as corn and sorghum.

Identification of ground cereal grains alone or in mixtures is a problem of control and regulatory laboratories of the feed, food, and other industries. Because cereal grains are similar in chemical constitution, identification is made microscopically.

Some microscopic characteristics of cereal grain starches that have proved sufficiently distinctive to be useful in identification of the grains in mixtures are presented. Attention is limited to the starches of the common commercial grains—viz., corn, wheat, grain sorghum, barley, oats, rye, and rice. Much