

R62-66

Spectral Examination of Cured-Meat Pigments During Frankfurter Processing^a

C. G. SIDWELL,^b HAROLD SALWIN,^c MARGARET DRIVER, AND
ROBERT B. KOCH^d

Quartermaster Food and Container Institute for the Armed Forces, US Army
Chicago 9, Illinois

(Manuscript received August 3, 1961)

TECHNICAL LIBRARY
U. S. ARMY
NATICK LABORATORIES
NATICK, MASS.

SUMMARY

Spectrophotometric analyses of aqueous-acetone extracts of frankfurters were useful for characterizing the porphyrin pigments that formed during smokehouse treatment or laboratory heating. The absorption curves showed differences among samples in spectral structure, rates of pigment development, and pigment stability. They also showed promise of differentiating among samples on the basis of storage stability.

Frankfurters are an important and popular food in the armed forces of the United States. Providing a high-quality product in remote overseas areas requires a minimum freezer-storage life of six months. Even though all frankfurters are produced in accordance with the requirements of the Federal Specification (1954) and appear identical when freshly prepared, their shelf life varies. Experience indicates that some lots exceed minimum stability needs while others become rancid within two months. It would therefore be desirable to develop objective methods capable of differentiating among lots on the basis of their predisposition to become rancid. For this purpose, our attention was directed to a study of the cured-meat pigments of frankfurters.

The heme pigments of uncured meat catalyze the oxidation of lipids (Watts, 1954; Younathan and Watts, 1959). Cured meats

are more stable (Younathan and Watts, 1959), but evidence on the relation of cured-meat pigments to lipid oxidation is less definite. Bauernfiend *et al.* (1954) and Wiesman and Ziemba (1946) showed that flavor stability paralleled pigment stability during freezer storage of such comminuted cured meat products as wieners and sausages.

Heme pigments are present in cured meat products principally as nitrosohemochrome (the reduced or ferrous form), as brown denatured metmyoglobin (the oxidized or hemichrome form), or as a mixture of the two. Greenish discoloration can result from advanced oxidation, which ruptures the porphyrin ring to form verdohemochromes (Am. Meat Inst. Foundation, 1960; Brown and Tappel, 1958). The globin portion of the pigment may be present in either the undenatured or denatured form, depending on the extent of heat processing.

Younathan and Watts (1959) hypothesized that the oxidized form of the pigment is the active catalyst in the oxidation of the fat. However, this cause-and-effect relation may at times be reversed because other changes accompanying the development of the cured-meat pigments during processing and their role have not been fully assessed. Brown and Tappel (1958) reported that nitrosohemochrome forms in many reducing media but that it is most stable when formed in the presence of sulfhydryl groups released

^aThis paper reports research conducted by the Quartermaster Food and Container Institute for the Armed Forces and has been assigned No. 2136 in the series of papers approved for publication. The views or conclusions are those of the authors, and are not to be construed as necessarily reflecting the views or indorsement of the Department of Defense.

^bPresent address: Veterans Administration, West Side Hospital, Chicago, Illinois.

^cPresent address: Food and Drug Administration, Washington, D.C.

^dPresent address: Minneapolis-Honeywell Regulator Company, Hopkins, Minnesota.

by heat denaturation of the protein. Metmyoglobin may therefore be formed in the presence of oxidized fat when there is a deficiency of sulfhydryls. Insolubilization of the pigment protein by heat may also retard interactions with the fat in the meat emulsion. Thus the physical state of the emulsion as well as its chemical composition may be a controlling factor in stability.

This paper reports the changes in the absorption spectra of the pigments as they develop under smokehouse and laboratory conditions. Some observations were also made on the relation of spectral characteristics to stability, but a more intensive study will be the subject of future work.

EXPERIMENTAL

Materials. Two local processors of frankfurters furnished samples withdrawn from the smokehouse at approximately one-hour intervals during the smoking treatment. They also provided unsmoked samples. The smokehouse temperature and the product internal temperature were recorded at each withdrawal (Table 1).

Table 1. Processing temperatures (°F) of Company A and Company B frankfurter samples.

Process- ing time (min)	Company A		Company B	
	Smoke- house temp.	Prod- uct temp.	Smoke- house temp.	Prod- uct temp.
0	60
60	160	122	130	130
90	140	135
120	160	152	150	140
180	180	155	150	146

The product of company A contained equal portions of beef and pork and was part of a military procurement under the Federal Specification (1954). The product of company B was 60% beef and 40% pork and was processed for the commercial market. The samples contained no added color or ascorbic acid. The samples were delivered to the Institute laboratories on the day prepared, stored overnight at 4.4°C, and tested the following day.

Methods. Sample preparation. After the casings were removed the frankfurters were ground through a food chopper and further triturated with a mortar and pestle. Spectral analyses and protein solubility tests were conducted on portions

of the prepared material, directly, and also after laboratory heating.

Laboratory heating. Twenty-gram samples of prepared frankfurters were wrapped and sealed in flat aluminum-foil packets of approx 3 × 5 × 3/16 in. The packets were immersed in a 60°C water bath for periods up to 3 hr. Above 60°C, the color developed too rapidly for study. Individual packets were withdrawn at intervals and the entire contents were tested.

Absorption spectra. Hornsey (1956) and others (Siedler and Schweighert, 1959; Tappel, 1957) used acetone-water solutions (40:3) to extract cured-meat pigments for spectral analysis. The extracted pigment, no longer attached to the globin moiety, was shown to be a nitrosoheme-acetone complex (Hornsey, 1956). It has also been hypothesized that the heme component probably exists in the meat unattached to the globin (Am. Meat Inst. Foundation, 1960).

In our work, the sample size was increased over that used by Hornsey and others in order to reveal the spectral structure more clearly. All extractions were conducted in subdued light to avoid light-catalyzed oxidation of the acetone complex (Tappel, 1957). Twenty grams of the prepared sample in a mortar were covered with 43 ml of the aqueous acetone. The sample was squeezed and pressed with a pestle for 5 min, care being taken to avoid undue aeration. The extract was filtered through Whatman No. 1 filter paper. Absorption spectra from 450 to 700 mμ were then obtained with a Model 14 M Cary recording spectrophotometer (mention of commercial products does not imply endorsement by the Department of Defense over other similar products not mentioned).

Soluble protein. A modification of Coretti's (1957) method was used to judge the extent of protein denaturation as an index of heat treatment during processing. Five-gram samples were extracted by intermittent shaking for 30 min in a flask with 25 ml of distilled water and filtered through Whatman No. 42 filter paper. Ten-ml portions of the filtrates were heated 15 min at 65°C to coagulate the soluble protein. The absorbancy of the turbid solution was measured with an Evelyn colorimeter with the 515 filter.

Keeping quality. Frankfurters in their cellulose casings were wrapped, 6 to a package, in aluminum foil and stored at -18°C in cardboard cartons. After 4-12 months of freezer storage, a frankfurter from each processing treatment was cooked 10 min in boiling water in a covered beaker. The intensity and character of the odor of the hot frankfurter samples were judged by a panel of laboratory personnel.

RESULTS AND DISCUSSION

Heretofore, aqueous-acetone extraction has been used largely for quantitative determination of cured-meat pigments. The purpose here was to observe qualitative and quantitative differences in spectra as they developed during processing or laboratory treatment.

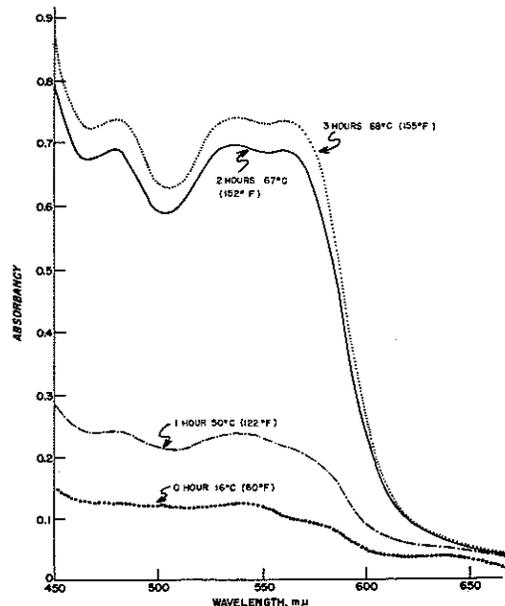


Fig. 1. Development of cured-meat pigment in Company A samples during smoking.

Pigment spectra of company A samples.

Fig. 1 shows the pigment spectra of samples removed from the smokehouse at hourly intervals. The indicated temperatures are internal sample temperatures. The absorption maxima at 480, 540, and 565 mμ, characteristic of nitrosohemochrome (Hornsey, 1956; Tappel, 1957), were fully developed in approx 2 hr when the internal temperature reached 67°C. Most of the color developed during the second hour of smoking.

Fig. 2 shows that the same characteristic spectrum developed during laboratory heating of the unsmoked frankfurters at 60°C. The small dimensions of the aluminum-foil packet permitted rapid heat penetration and faster development of color.

Pigment spectra of company B samples.

The pigment in samples from company B developed in a different manner from that

described above. Pigment concentration was near a maximum after only 1 hr of smokehouse treatment when the internal temperature was 54°C. The curves in Fig. 3 gave evidence of instability upon heating in the smokehouse beyond the first hour. The absorption maximum at 565 mμ became progressively less well-defined until it disappeared, at 3 hr. Concurrently, a peak developed at 635 mμ, suggestive of a hemichrome form of the pigment.

Fig. 4 shows the results of laboratory heating of the unsmoked frankfurters at 60°C. Under these conditions, as in the smokehouse, the pigment developed more rapidly than it did in company A samples. In other respects, however, the pigment characteristics were more like those of Figs. 1 and 2 than those of Fig. 3. The spectral structure was that of nitrosohemochrome, and stability was good upon continued heating for 3 hr. The results in Figs. 3 and 4 indicated that the meat emulsion and the curing ingredients were inherently capable of developing the stable nitrosohemochrome, but that a smokehouse or other processing factor led to instability.

The fact that the same procedure was used for obtaining the data in Figs. 3 and 4 indi-

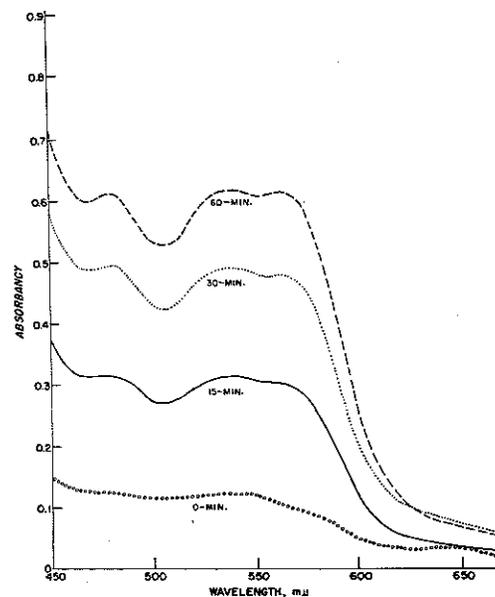


Fig. 2. Development of cured-meat pigment in unsmoked Company A samples during laboratory heating at 60°C.

PIGMENT CHANGES DURING FRANKFURTER PROCESSING

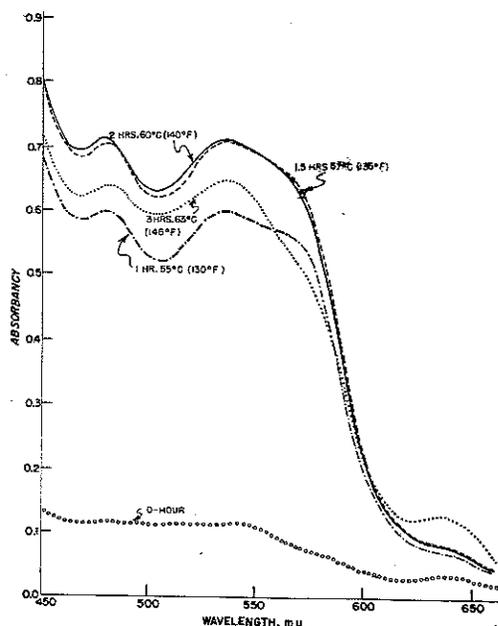


Fig. 3. Development of cured-meat pigment in Company B samples during smoking.

cated that the 635-m μ peak of Fig. 3 was not the result of light-catalyzed oxidation of the extracts. Extracts of the smoked samples did not develop the 635-m μ peak when exposed to subdued light for 2 hr. Furthermore, Hornsey (1956) showed that the light-catalyzed oxidation product had a single absorption maximum, at 566 m μ .

Samples of the material smoked for 3 hr were subjected to laboratory heating at 60°C. The absorption curves in Fig. 5 show that the spectral shift from 565 to 635 m μ that occurred during smoking could be reversed by the laboratory heating. Total exclusion of air under these conditions suggested that the smokehouse may not have had a sufficiently reducing atmosphere to prevent oxidation of the nitrosohemochrome (Fox, 1959).

Absorbancy index of frankfurter pigment. The sharpness of the α -band in the 560-m μ region is characteristic of the hemochrome structure (Lemberg and Legge, 1949). An absorbancy index employing the measurements at 505, 540, and 565 m μ may therefore be useful for characterizing the extracted pigment. Table 2 shows the values

Table 2. Absorbancy index of frankfurter pigment.

$$\frac{A_s 565 \text{ m}\mu - A_s 505 \text{ m}\mu}{A_s 540 \text{ m}\mu - A_s 505 \text{ m}\mu}$$

Smoking time, or lab. heating time (min)	Company A samples		Company B samples		
	Smoked	Un-smoked, lab. heated	Smoked	Un-smoked, lab. heated	
15	0.67	0.94	-1.90
30	0.80	0.94	0.00
60	-0.18	0.94	0.59	0.94	0.53
90	0.47
120	0.91	0.32	1.00
180	0.97	-1.20	1.00

of this index $\frac{A_s 565 \text{ m}\mu - A_s 505 \text{ m}\mu}{A_s 540 \text{ m}\mu - A_s 505 \text{ m}\mu}$ for the

spectra in Figs. 1 to 5. They ranged from 1.00, for nitrosohemochrome; to -1.90, for oxidized pigment.

Relation of temperature during smoking to pigment development. The effect of temperatures on pigment development, independent of other factors, could not be ascertained from these experiments. The greatest pigment concentration (Fig. 1) and highest product temperature (68°C, 155°F, Table 1)

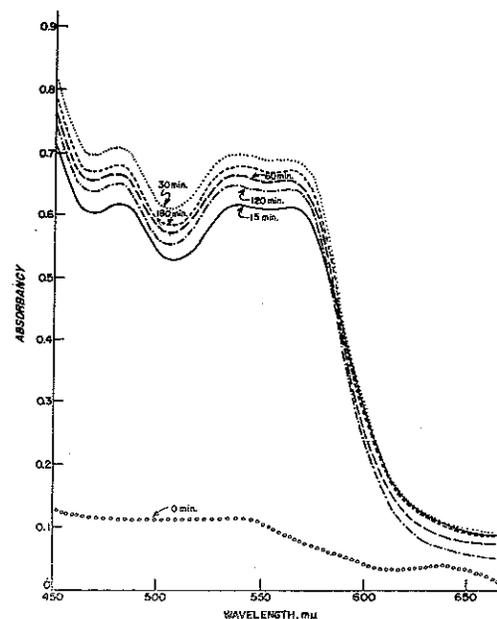


Fig. 4. Development of cured-meat pigment in unsmoked Company B samples during laboratory heating at 60°C.

were attained in the company A smokehouse. The concentration could not be equaled by 60 min of laboratory heating at 60°C (Fig. 2). The highest product temperature reached in the company B smokehouse was 63°C. The unstable pigment that developed could be equaled in concentration by 30 min of laboratory heating (Figs. 3 and 4). The greater stability of the pigment developed in the company A product appeared to be related to the higher product temperatures reached at the end of 120 and 180 minutes of smoking. The final product temperature prescribed by the Federal Specification (1954) for frankfurters is 66-71°C (150-160°F). A second source (Am. Meat Inst. Foundation, 1960) considers 66°C to be a minimum end-point temperature but recommends a temperature of 68°C to provide a safety margin for any variation that may arise from position in the smokehouse or errors in thermometer placement. This higher processing temperature is said to provide better keeping quality in the product.

The processing temperatures in Table 1 and the pigment spectra in Figs. 1 and 2 suggest that a product temperature of 68°C is not a required minimum for pigment de-

velopment but is important for conferring stability on the pigment that develops at lower temperatures.

Relation of protein solubility to pigment formation and stability. A close relation between pigment formation and stability and the extent of protein denaturation has been reported (Am. Meat Inst. Foundation, 1960; Fox, 1959; Siedler and Schweigert, 1959). The protein-solubility results on the company A samples (Table 3) showed a consistent pattern with time of smoking. Complete protein denaturation as defined by loss of solubility occurred sometime between the first and second hours of smoking. At the same time a definite increase in pigment concentration occurred (Fig. 1) and the absorbancy index reached 0.91 (Table 2).

The smoking time for minimum solubility in the company B samples also coincided with the interval of rapid pigment development—approx 1 hr. However, results of the solubility test on the smoked samples were not entirely consistent with processing time. Although sample variation may be partly responsible, there may be a relation between the erratic behavior of this product and the instability of the pigment.

Relation of pigment development to keeping quality. Sensory evaluations were made after storage at -18°C. A rank order of stability was assigned that represented degree of retention of normal frankfurter odor in the cooked samples. Throughout 12 months of storage the stability of company A samples was directly related to processing time, pigment concentration, absorbancy index, and

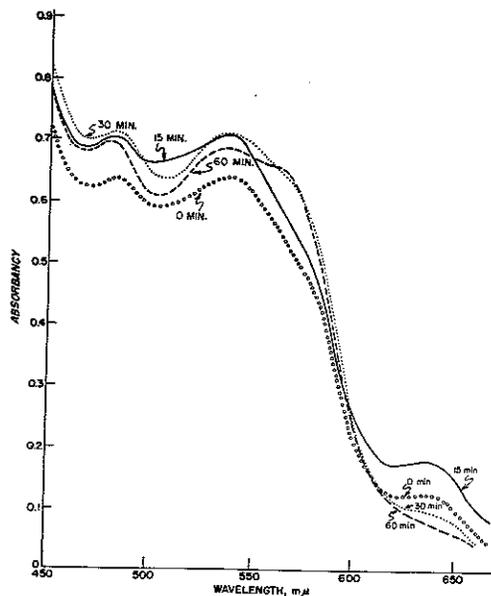


Fig. 5. Reversal of 565-635 mμ spectral shift (3-hr sample, Fig. 3) by additional laboratory heating at 60°C.

Table 3. Soluble protein test on frankfurters (absorbancy, A_s 515 filter).

Smoking time, or lab. heating time (min)	Company A samples		Company B samples
	Smoked	Smoked	Lab. heated
0	0.429 ^a	0.301 ^a	0.301 ^a
15	0.143
30	0.137
60	0.296 ^a	0.027	0.056
90	0.375
120	0.080	0.029	0.061
180	0.017	0.127

Absorbancy of 0.080 or lower represents negligible turbidity.

^a Test conducted on 1-g rather than 5-g sample.

insolubility of the protein. From the sixth to the twelfth months, only the samples smoked for 2 or 3 hr had a normal frankfurter odor. All of the smoked samples retained good color for 12 months. Among company B samples, which were more erratic in their pattern of pigment development, only the sample smoked 2 hours retained an odor resembling that of normal frankfurters after 4 months of storage. Off-odors were detectable in this sample after 6 months, and severe greenish discoloration occurred in all samples after 10 months. Storage stability of the company B product was definitely inferior to that of the company A product.

REFERENCES

- American Meat Institute Foundation. 1960. "The Science of Meat and Meat Products." W. H. Freeman & Co., San Francisco and London.
- Bauernfiend, J. C., E. G. Smith, and G. K. Parman. 1954. Ascorbic acid betters color, flavor of meat. *Food Eng.* **26** (6), 80.
- Brown, W. D., and A. L. Tappel. 1958. Pigment-oxidation relationships to meat-color stability. *Proc. Tenth Research Conference, Am. Meat Inst. Foundation.*
- Coretti, K. 1957. A rapid method to demonstrate that canned ham was heated satisfactorily. *Die Fleischwirtschaft* **3**, 114.
- Federal Specification. 1954. Frankfurters; Chilled and Frozen, PP-F-660.
- Fox, J. B., Jr. 1959. The pigment of cured meats. *Am. Meat Inst. Foundation Bull.* No. 41.
- Hornsey, H. C. 1956. The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. *J. Sci. Food Agr.* **7**, 534.
- Lemberg, R., and J. W. Legge. 1949. "Hematin Compounds and Bile Pigments." Interscience Publishers, New York.
- Siedler, A. J., and B. S. Schweigert. 1959. Effects of heat, nitrite level, iron salts, and reducing agents on formation of denatured nitrosomyoglobin. *J. Agr. Food Chem.* **7**, 271.
- Tappel, A. L. 1957. Spectral studies of the pigments of cooked cured meats. *Food Research* **22**, 479.
- Watts, B. M. 1954. Oxidative rancidity and discoloration in meat. "Advances in Food Research" **5**, 1.
- Wiesman, C. K., and J. V. Ziemba. 1946. How to prevent rancidity in frozen pork sausage. *Food Inds.* **18**, 1863.
- Younathan, M. T., and B. M. Watts. 1959. Relationship of meat pigments to lipid oxidation. *Food Research* **24**, 728.