6:9 Synthesis of dinitrosyl ferrohemochrome and its characteristics

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Introduction

Sodium nitrite, in view of its multifunctional role, is perhaps the most important ingredient in meat curing processes. Its effects on the chemical and organoleptic properties as well as the oxidative and microbial stability of cured meats has been thoroughly investigated (Shahidi et al., 1984). Unfortunately nitrite is also responsible for the formation of N-nitrosamines in cured meats and may also lead to the formation of these compounds in the stomach (Gray et al., 1982). N-nitroso compounds such as those of pyrrolidine and dimethylamine have been shown to be carcinogenic in experimental animals and a recent study by Newberne and coworkers of the Massachusetts Institute of Technology has suggested that nitrite itself may produce cancer when fed in high doses to rats (Newberne, 1979). However, in this connection see the report called "Re-evaluation of the pathology findings of studies on nitrite and cancer" (Endicott et al., 1980).

In order to eliminate the formation of nitrosamines in cured products and in the gastric tract efforts have been made to reduce the amount of nitrite used in the curing process or to develop alternative methods of meat curing. It is not expected that any single substance could duplicate all the functions of nitrite, and for this reason we have focused our attention on a coordinated multi-component curing system. These systems are designed to contain a pigment reproducing the colour of nitrite-cured meats, an antioxidant/chelator system to retard the development of warmed-over flavour and rancidity, and an antimicrobial agent to ensure the safety of the products.

The colour of meat is due to the pigment myoglobin. Nitric oxide binds itself to myoglobin to produce the red-coloured nitrosomyoglobin. Upon heat processing, the reasonably stable pink colour characteristic of cured meats is formed. The chemistry of the pigment formed has been extensively studied (Livingston and Brown, 1981) and confirmed to be dinitrosyl ferrohemochrome, DNFH (Tarladgis, 1962; Lee and Cassens, 1976).

Complexes of myoglobin with ligands other than nitric oxide, such as nicotinic acid and its derivatives, as well as with pyridine and other nitrogeneous heterocyclic compounds, have been prepared (Howard et al., 1973), and their application to both fresh and cured meats patented. However, their use has not been adopted, since some had colours untypical of cured meats and, more importantly, due to the fact that these complexes are less stable in the presence of oxygen than the nitric oxide complex. Also their toxicological properties are questionable or unknown.

To reproduce the colour of cured meats we have made use of hemin, an iron III porphyrin prepared from beef red blood cells, to preform the actual cooked cured-meat pigment, dinitrosyl ferrohemochrome. Preparation of DNFH pigment from hemin and sodium nitrite was reported in a previous paper (Shahidi et al., 1984). The purity of the pigment so obtained was 65-72% and it did not impart a clean pink colour when applied to meat.
In this paper we report on our result for the preparation of the cooked cured-meat pigment, using nitric oxide as the nitrosating agent.

Materials and Methods

All chemicals and solvents used in these studies were reagent-grade commercial products except for hemin which was prepared from beef red blood cells as described previously (Shahidi et al., 1984). Absorption spectra were recorded using a Beckman DU-7 spectrophotometer.

Dinitrosyl Ferrohemochrome Synthesis

In a volumetric flask, hemin (0.030 mmol) was dissolved in enough 0.04 M sodium carbonate solution to reach the 10 ml mark. While in darkness and under nitrogen, 0.5 ml of this solution was added to a centrifuge tube containing a reductant (0.05 - 0.15 mmol) and 9.5 ml of 0.2 M (unless otherwise specified) acetate buffer, pH 4 to 11. Then gaseous nitric oxide was bubbled into the mixture and the tube was capped and kept in the dark. On centrifugation of the mixture, the reddish pigment settled out. The supernatant was poured off and the solids washed with a 0.02% ascorbic acid solution to ensure the removal of nitric oxide and other water-soluble materials. Reductants used in these studies were ascorbic and isoascorbic acids as well as sodium ascorbate and sodium dithionite. In all cases the visible absorption spectra of the pigment in 80% aqueous acetone (Hornsey, 1956) were then recorded.

Analysis of the pigment was performed spectrophotometrically by conversion of all porphyrin materials to acid hematin, following the standard procedure of Hornsey (1956).

Stability of the pigment so obtained in aqueous solutions as a function of storage time was monitored under various conditions. Four sets of centrifuge tubes containing the pigment were used in these experiments. The first set was kept in darkness and under a nitrogen atmosphere with a slight positive pressure of nitric oxide. The second set was placed between two 35-watt fluorescent lights in the absence of oxygen. The third set was kept in the dark, but open to air. Finally, the last set was kept in light as above and also open to air. At different time intervals one tube of each set was removed and after centrifugation and usual work up, as described above, the visible spectrum of the pigment in 80% aqueous acetone was recorded.

Results and Discussion

The visible spectrum for the cooked cured-meat pigment, dinitrosyl ferrohemochrome, prepared by the reaction of hemin with nitric oxide and that of the extracted pigment from a commercial ham, in 80% acetone-water solution are shown in Figure 1. As expected, the absorption pattern of these pigments is identical.

Among the reducing agents used for the preparation of the DNFH pigment, sodium ascorbate was the most effective as indicated by the larger absorbance of the pigment at 535 nm. Ascorbic and erythorbic acids were slightly less effective, and sodium dithionite was the least effective reductant (Table 1). The yield of dinitrosyl ferrohemochrome as a function of the pH of the acetate buffer was monitored. Generally, a better yield of DNFH was obtained at higher pH of the solutions. However, at pH 6.5, the buffer was slightly more effective than that at pH 7.0 (Table 2). Solutions with pH > 9 gave pigments which showed a relatively high absorbance at 535 nm; however, they were very unstable and did not survive even short periods of storage. They gave rise to green-coloured solutions after 24 h in darkness and in the absence of air.

Although pigments prepared in the pH range of 5.5-7.0 survived for several months when kept in sealed tubes under a nitrogen atmosphere with a slight positive pressure of nitric oxide, in ordinary polypropylene centrifuge tubes capped with plastic lids they survived only for 5 weeks when stored in darkness and only for 3 weeks in the presence of light, likely due to the diffusion of air into the tubes. Both light and oxygen accelerated the deterioration of the pigment, and decomposition began after 4-6 h when kept in the presence of both light and air, or 30-36 h in air and in the dark.

While the absorbance of the 563 nm peak for the freshly prepared pigment is always larger than that at 535 nm (in 80% acetone-water solution), upon standing the peak at 563 nm starts to shrink and becomes smaller than that at 535 nm. Pigment extracted from old samples of commercial ham showed the same characteristic pattern. Upon further standing, the 563 nm peak disappears and, at this point, the solution is no longer red in colour and the 535 nm peak has also shrunk (Figure 2). Further standing results in the disappearance of the 535 nm peak and the solution turns green in colour.

Variation of the concentration of pH 6.5 acetate buffer (0.05, 0.10, 0.20, and 0.50 M) had little effect on the yield of the DNFH pigment, as indicated by the absorbance at 535 nm (Table 3).

Spectrophotometric analysis of the cooked cured-meat pigment obtained by our best procedure in which sodium ascorbate was used as the reductant in 0.2 M pH 6.5 acetate buffer indicated a purity of > 97% (Hornsey, 1956).

The dinitrosyl ferrohemochrome prepared in this fashion imparted a clean pink colour to comminuted meats, when used in place of sodium nitrite. It is interesting to note that only 1 ml beef red blood cells is sufficient to prepare enough pigment to reproduce the characteristic colour of nitrite-cured meats in 1 kg of pork. Further work is in progress to produce the pigment on a larger scale and to develop a practical procedure suitable for its application to both comminuted and solid cuts of meat on a commercial scale.

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TABLE 1
The effect of reductant on the yield of DNFH as indicated by the intensity of its 535 nm absorption peak.

Reductant	Absorbance
Sodium ascorbate	0.3583
Ascorbic acid	0.3276
Isoascorbic (Erythorbic) acid	0.3172
Sodium dithionite	0.2517

TABLE 2
The effect of pH of 0.2 M acetate buffer on the yield of DNFH as indicated by the intensity of its 535 nm absorption peak.

pH	Absorbance	Appearance after 24 h
4.0	0.2776	slightly reddish solution with precipitate
5.5	0.3009	slightly reddish solution with precipitate
6.5	0.3319	red solution with precipitate
7.0	0.3035	red solution with precipitate
9.0	0.3371	green solution with precipitate
11.0	0.3448	green solutions

TABLE 3

The effect of concentration of pH 6.5 acetate buffer on the yield on DNFH as indicated by the intensity of 535 nm absorption peak

Buffer concentration	Absorbance
0.05	0.3210
0.1	0.3262
0.2	0.3460
0.5	0.3366

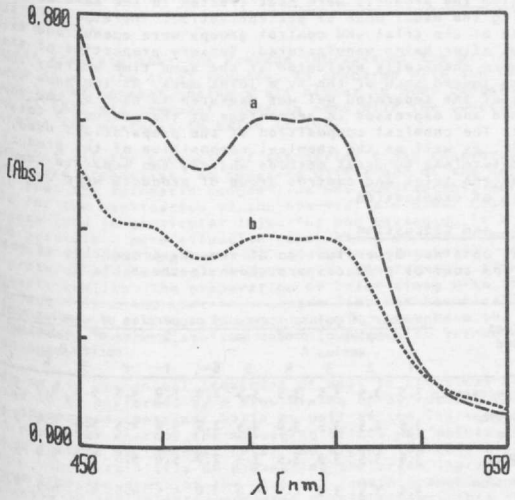

Figure 1. Absorption pattern of DNFH pigment in 80% acetone-water, a) preformed pigment, b) pigment extracted from commercial ham.

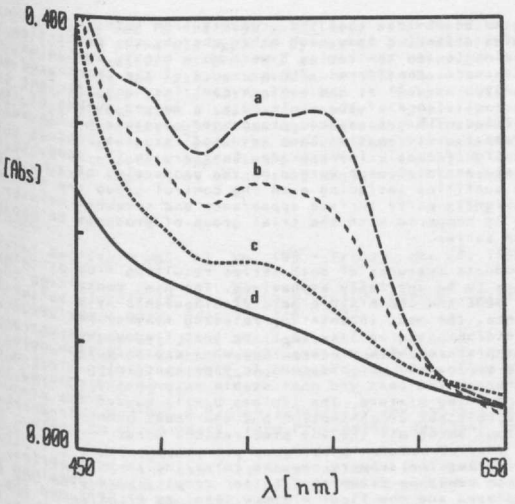

Figure 2. Progressive deterioration of DNFH pigment, a) freshly prepared, b) and c) partially decomposed, d) fully decomposed.