Alternative Meat-Curing Systems. I. Cooked Cured-Meat Pigment

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ABSTRACT

In an effort to develop alternative meat-curing systems to substitute for the common curing agent sodium nitrite, we have investigated the preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome. Hemin, the precursor to this synthetic pigment was prepared from beef red blood cells using modified versions of the methods described by Schalfejeff or Labbe and Nishida. About 80-99 percent of the blood's hemin can be extracted using these modified methods of preparation. Dinitrosyl ferrohemochrome was prepared by the reaction of hemin with sodium nitrite or nitric oxide and a reductant in buffered solutions. Application of this pigment to comminuted meat produced a pink colour indistinguishable from that of nitrite-cured samples. Further work on the preparation and application of this pigment is underway. Studies on the control of oxidative rancidity, which apparently masks the cured-meat flavour, are in progress using ground meat, both with and without the "synthetic" pigment.

INTRODUCTION

For over five decades nitrite has been used in the curing of meat products (Rubin, 1977). Nitrite has multifunctional properties. It reacts with the muscle-protein, myoglobin, to produce the characteristic cured-meat colour (Giddings, 1977) and gives the typical flavour to cooked meats such as ham (Gray et al., 1981). Nitrite also acts as an antioxidant (MacDonald et al., 1980) by preventing the formation of warmed-over flavour and plays a predominant role in inhibiting Clostridium botulinum growth and toxin formation (Hauschild et al., 1982; Pierson and Smoot, 1982).

Recently, nitrite has become the source of some very serious concerns due to its reaction with amines and amino acids found in meats to produce small amounts of nitrosamines (Gray et al., 1982), some of which like N-nitrosopyrrolidine and N-nitrosodimethylamine have been found to be carcinogenic in experimental animals. Although nitrosamines are not generally present in the cured raw products, they are almost always present in bacon after frying. The residual nitrite present in cured meats may also lead to the formation of nitrosamines in the stomach. In order to eliminate the formation of nitrosamines in cured products, efforts have been made to reduce the amount of nitrite and to develop alternative methods of meat curing (Sebranek, 1979).

Although no suitable substitute has yet been found for nitrite, the Canadian Government remains committed

to the removal of nitrite from all cured meat products if and when such replacements are found (Holley, 1981).

Since it is unlikely that a single compound will be found that can perform all the functions of nitrite, the present program is aimed at developing a multicomponent curing system that will duplicate each of the key properties of nitrite individually. The program will be carried out in three phases.

In the first phase we are working on the development of an alternative method for producing the cooked cured-meat colour. In the second phase, we plan to develop an antioxidant and/or sequestrant system to perform antioxidant functions of nitrite, thus controlling oxidative rancidity in meat products which we believe to be closely related to the production of the cured-meat flavour. The anti-microbial activity of the curing system will be developed under a separate program by a research group in Agriculture Canada.

In this paper we report on our results for the preparation of hemin, a precursor to cooked cured-meat pigment, dinitrosyl ferrohemochrome (DNFH), and subsequent preparation of DNFH from it using sodium nitrite as the nitrosating agent (Figure 1). The classical acetic acid method of Schalfejeff (1885) and the procedure of Labbe and Nishida (1957) have been adapted for preparation of hemin.

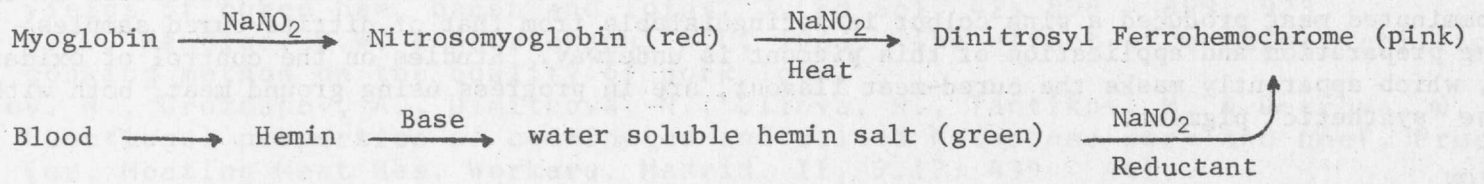


Figure 1. Reaction of myoglobin and hemoglobin with $NaNO_2$.

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 obtained from Canada Packers, Toronto Plant. Visible absorption spectra were recorded using a Backman DU-7 and a Perkin Elmer 703 atomic-absorption instrument was used for iron determinations. Kjeldahl determinations of nitrogen were performed using a Büchi 425 digestor and a Büchi 320 nitrogen distillation unit.

Preparation of Hemin from Beef Red Blood Cells

A. Glacial acetic acid (Schalfejeff) method

In a 2 L beaker, 600 mL glacial acetic acid saturated with sodium chloride were heated and maintained at a temperature of 90-100°C. One hundred mL red blood cells containing different amounts of water were slowly

in 100 mL red blood cells (1.308 g), were obtained when 300 or 400 mL water were added.

The yield and purity of the hemin from modified versions of the Labbe-Nishida method under different experimental conditions (using either strontium or calcium chloride) are reported in Table 2. Additional experiments were also carried out at a red-blood-cell to extraction-solution ratio of 1:24 in which the extraction solutions were prepared from acetic acid and acetone in the ratios of 1:1 and 1:2. These latter results are footnoted to Table 2.

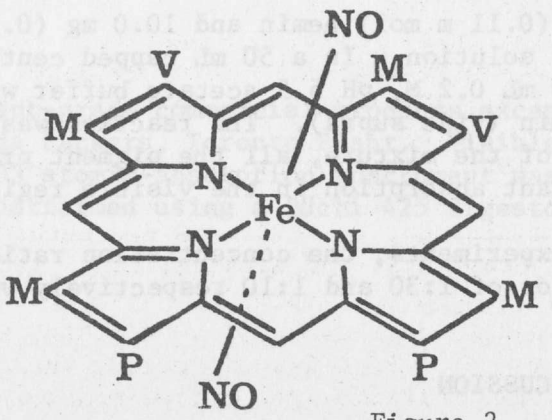
Yields as high as 84% were obtained when red blood cells were added to extraction solutions at ratios of 1:27, 1:30, 1:33 and 1:36 (V:V). These yields are nearly double those obtainable by the original Labbe and Nishida method in which red blood cells are diluted with water before their addition to acetic acid-acetone-$SrCl_2$ solution. For strontium chloride, an acetic acid to acetone ratio of 1:4, rather than the 1:3 indicated in the original method, gave the best yields. Dilution of the red blood cells with water, as described by Labbe and Nishida (1957), adversely affected the yields and was abandoned in preliminary experiments.

The effect of decreasing the amount of either calcium or strontium chloride salts from 2% to 1% is shown in Table 3. In all cases this resulted in a reduction of the yield and the purity of hemin.

The spectrophotometric analysis of hemin as its bipyridine ferrohemochrome showed that the absorbance ratio of the α-peak (557 nm) to the β-peak (526 nm) was 1.93 and the ratio of the Soret peak (418.5 nm) to α-peak was 5.2. These compare well with the literature values of 1.96 and 5.57 respectively (Falk, 1964).

The observed iron content of the hemin samples obtained from both methods of preparation corresponded well with the value of 8.58% calculated on the basis of the formula $C_{34}H_{32}ClFeN_4O_4$, indicating that the hemin isolated in these studies was of high purity (Tables 1 and 2). While under optimum conditions both methods were equally efficient in extracting hemin from red blood cells, the acetic acid method has the advantage of being quick and more suitable for preparation of small quantities of hemin. The acetic acid-acetone-salt system is more elaborate and time consuming; however, it could be adapted more easily for bulk preparation of hemin.

In the synthesis of dinitrosyl ferrohemochrome (Fig. 2), a large molar excess of both sodium nitrite (∿10 fold) and reductant (∿30 fold) was necessary. This is comparable to commercial meat curing processes where sodium nitrite and sodium ascorbate or erythorbate are used (Tables 4 and 5).



M: CH_3
V: $CH{=}CH_2$
P: $CH_2CH_2CO_2H$

Figure 2.

Spectrophotometric analysis of this dinitrosyl ferrohemochrome in 80% acetone solution (Hornsey, 1956) indicated a purity of 65 to 72%. The nitrogen content of this synthetic pigment, as determined by Kjeldahl analysis, showed a purity of 68%, and is in accord with the results obtained spectrophotometrically.

The pigment, in its present state of purity, does not impart a clean pink colour when applied to meat. However, pigments which are over 90% pure have since been prepared and produce the characteristic cooked cured-meat colour. Further studies are in progress to improve the yield and purity of dinitrosyl ferrohemochrome.

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Table 1. Yield and purity of hemin prepared by the glacial acetic acid method.

Red blood cells (mL)	Water (mL)	Hemin[a] (mg)	Fe (%)	Purity (%)
100	0	1100 ± 118	8.43	98.2
100	100	1108 ± 60	8.49	98.9
100	200	1166 ± 54	8.56	99.8
100	300	1260 ± 116	8.58	100.0
100	400	1296 ± 14	8.33	97.1

[a] Average of 6 replicates with standard deviations from mean.

Table 2. Yield and purity of hemin in the acetone-acetic acid-salt method.[a]

Ratio of red blood cells to extraction solution	Acetic Acid to Acetone Ratio								
	1:3			1:4			1:5		
	Yield(mg)	Fe(%)	Purity (%)	Yield (mg)	Fe(%)	Purity	Yield (mg)	Fe(%)	Purity (%)
Salt = Strontium chloride hexahydrate ($SrCl_2,6H_2O$)									
1:15	138±24	8.41	98.0	-	-	-	-	-	-
1:18	273±6	8.47	98.7	-	-	-	-	-	-
1.21	528±80	8.49	98.9	-	-	-	-	-	-
1:24[b]	685±90	8.58	100.0	935±62	8.56	99.8	465±34	8.57	99.9
1:27	834±109	8.58	100.0	744±32	8.56	99.8	599±41	8.57	99.9
1:30	870±100	8.58	100.0	1072±16	8.44	99.0	798±38	8.30	96.7
1:33	944±14	8.58	100.0	1069±18	8.49	98.4	987±47	8.29	96.6
1:36	933±12	8.58	100.0	1042±71	8.51	99.2	978±31	8.58	100.0
1:45	862±8	8.58	100.0	868±10	8.56	99.8	845±95	8.41	98.0
Salt = Calcium Chloride ($CaCl_2$)									
1:15	136±52	8.48	98.8	-	-	-	-	-	-
1:24[c]	637±90	8.58	100.0	820±145	8.58	100.0	954±40	8.56	99.8
1.27	1109±25	8.26	96.3	1051±36	8.25	96.2	883±29	8.58	100.0
1:30	1124±3	8.58	100.0	963±54	8.29	96.6	980±64	8.43	98.3
1:33	1092±37	8.28	96.5	1069±16	8.44	98.4	912±29	8.58	100.0
1:36	1020±21	8.28	96.5	990±13	8.47	98.7	1043±42	8.29	96.6
1:45	959±50	8.53	99.4	808±38	8.58	100.0	776±34	8.53	99.4

[a]100 ml red blood cells were used in all cases. Yields are average of 3 to 6 experiments and standard deviations from means are given.

[b]Acetic acid:acetone ratio of 1:1 gave a yield of 528±81 and a purity of 97%, and a ratio of 1:2 gave a yield 206±68 and a purity of 95.5 %.

[c]An acetic acid:acetone ratio of 1:1 a yield of 520±72 and a purity of 98%, and a ratio of 1:2 gave a yield of 199±58 and a purity of 97.1%.

Table 3. Effect of concentration of salts on the yield and purity of hemin by the acetone-acetic acid-salt method.

Salt[a]	Hemin (mg)	Fe (%)	Purity (%)
$SrCl_2.6H_2O$ (2%)	685 ± 90	8.58	100.0
$SrCl_2.6H_2O$ (1%)	401 ± 36	8.27	96.4
$CaCl_2$ (2%)	637 ± 90	8.58	100.0
$CaCl_2$ (1%)	305 ± 30	8.29	96.6

[a] The volume ratio of acetic acid to acetone was 1:3 and that for red blood cells (100 mL) to extraction solution was 1:24.

Table 4. Effect of the concentration of sodium nitrite on the absorbance, A, of dinitrosyl ferrohemochrome solution at 540 nm[a].

$[NaNO_2]$, mM	$[NaNO_2]/[Hemin]$	A[b]
10.7	56	0.341
8.0	42	0.372
5.4	28	0.420
3.8	20	0.463
2.1	11	0.492
1.1	5.8	0.441
0.54	2.8	0.400

[a] Concentrations of hemin and sodium dithionite were 0.19 and 11.0 mM respectively.

[b] The higher the absorbance, the better the yield.

Table 5. The effect of the concentration of sodium dithionite on the yield of dinitrosyl ferrohemochrome[a].

$[Na_2S_2O_4]$, mM	$[Na_2S_2O_4]/[Hemin]$	A[b]
84.5	445	0.430
69.4	365	0.402
41.3	217	0.442
32.6	172	0.471
14.1	74	0.483
8.9	47	0.441
6.8	36	0.494
3.4	18	0.451

[a] Concentrations of hemin and sodium nitrite were 0.19 and 2.09 mM respectively.

[b] The higher the absorbance, the better the yield.