SYNTHESIS OF COOKED CURED-MEAT PIGMENT, DINITROSYL FERROHEMOCHROME, AND ITS COLOUR CHARACTERISTICS

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SUMMARY

The cooked cured.meat pigment, dinitrosyl ferrohemochrome (DNFH) was synthesized from bovine red blood cells and a nitrosating agent, in the presence of a reductant and in buffered solutions at elevated temperatures. Reductants employed were ascorbic acid (AA), erythorbic acid (EA) and/or ascorbyl palmitate (AP). Yields and purities of ± 94% were obtained in most cases. Colour quality of DNFH-treated meats after cooking was similar to that of nitrite.cured meats as indicated by their corresponding L, a, b values.

INTRODUCTION

The role of sodium nitrite in meat curing and its effect on colour. flavour, oxidative and microbial stability of processed meats has been thoroughly investigated (Shahidi et al. 1988). Our own approach has concentrated on the latter option. Since no other compound is known to perform the multi.functional role of nitrite, we have formulated multi.component mixtures which may be used as nitrite, we have formulated multi-component mixtures which may be used as nitrite alternatives in meat curing (Shahidi et al. 1988).

The colour of meat is due to the presence of myoglobin/ haemoglobin. Nitric oxide binds itself to myoglobin/ haemoglobin to produce the red-coloured nitrosomyoglobin/ nitrosohaemoglobin; upon heat treatment, the reasonably stable pink colour characteristic of cured meats is formed. The chemistry of the latter pigment has been studied and confirmed to be dinitrosyl ferrohemochrome (DNFH) (Fox, 1966; Lee and Cassens, 1976). We have also prepared this pigment from hemin and a nitrosating agent such as sodium nitrite or nitric oxide (Shahidi et al. 1984; 1985). Hemin was in turn prepared from bovine red blood cells. In this process. DNFH was produced quantitatively and with a high purity. Furthermore DNFH had some weak antioxidant properties (Shahidi et al. 1987). However. this process was somewhat laborious and used a large volume of organic solvents.

It is the objective of this paper to investigate preparation of DNFH directly from bovine red blood cells. Colour characteristics of the prepared DNFH, as such, or in DNFH.treated meats will be studied.

MATERIALS AND METHODS

All chemicals and solvents used in this study were food.grade or reagent.grade commercial products. Freshly collected bovine red blood cells were obtained from Canada Packers in Toronto and were stored in Whirl-Pak polyethylene bags and were transferred to St. John.s in frozen state. Prior to use, red blood cells were thawed overnight at 4°C.

Table 1. Yield and Purity of Dinitrosyl Ferrohemochrome under Different Preparation Conditions.[1]

Experiment No.	Method[2]	Yield[3] %	Purity[3] %
1	Control	ND	ND
2	AA, 1:5	ND	ND
3	EA, 1:5	ND	ND
4	AP, 1:5	51.9±1.0	96.4±1.6
5	(2)+(4)	58.6±8.7	97.8±0.5
6	(3)+(4)	44.8±6.9	95.3±1.0
7	AA, 1:10	94.0±1.7	99.0±0.6
8	EA, 1:10	94.5±2.0	99.0±0.6
9	AP, 1:10	59.7±2.4	97.1±1.0
10	(7)+(9), No NaOH	66.1±1.9	98.8±1.0
11	(7)+(9), 0.1M NaOH	83.5±1.8	99.4±0.6
12	(7)+(9), 0.2M NaOH	94.9±1.0	99.2±1.0
13	(7)+(9), 0.3M NaOH	88.6±2.0	97.0±2.0

[1] Nitrite concentration was 10 times the concentration of available hemin in the reaction mixture.

[2] Reducing agents were AA- ascorbic acid; EA- erythorbic acid; and AP- ascorbyl palmitate. Ratios of hemin to reductants were 1:5 or 1:10 as indicated.

[3] ND - not determined.

Table 2. Hunter L, a, b values of Cooked Ground Meats.

Experiment No.	Pork Type	Treatment[1]	Hunter Values L	a	b	Visual Colour
1	I	Control	46.9±0.2	6.6±0.1	11.4±0.2	dark brown
2	I	$NaNO_2$, 156 ppm	46.4±0.3	13.0±0.5	8.5±0.2	dark pink
3	I	DNFH, 12 ppm	46.3±0.2	11.6±0.2	8.4±0.2	dark pink
4	II	Control	59.1±1.02	5.1±0.1	11.8±0.1	brown
5	II	$NaNO_2$, 156 ppm	58.6±0.1	12.1±0.1	9.3±0.1	pink
6	II	DNFH,	56.7±0.1	12.3±0.2	9.2±0.2	pink
7	II	(6)+STPP, 3000 ppm	56.0±0.3	12.5±0.1	8.9±0.2	pink
8	II	(6)+STPP, 1500 ppm+ SAPP, 1500 ppm	56.3±0.1	13.5±0.1	8.9±0.1	bright pink
9	III	Control	62.7±0.3	4.1±0.1	11.4±0.1	light brown
10	III	$NaNO_2$, 156 ppm	61.1±0.1	11.3±0.1	9.8±0.1	light pink
11	III	DNFH, 12 ppm	60.3±0.3	11.6±0.1	9.4±0.1	light pink

[1] Symbols are: DNFH - dinitrosyl ferrohemochrome; STPP - sodium tripolyphosphate; SAPP - sodium acid pyrophosphate.

Absorption spectra were recorded using a Beckman DU.70 or a Shimadzu UV.260 spectrophotometer. A Perkin-Elmer 2380 atomic absorption spectrophotometer was used to determine the iron content of the red blood cells in order to estimate their hemin level. A Gardner XL20 colorimeter was used to determine colour parameters, L for lightness, a for redness. and b for yellowness.

Preparation of Dinitrosyl Ferrohemochrome (DNFH)

Bovine red blood cells (approximately 10 g) were added to a 90 mL solution consisting of water: 0.2M NaOH (unless otherwise specified see Table 1) at a ratio of 8:1 (v/v).

The latter solution contained sodium nitrite and reductant(s). The ratios of the available hemin to reductant and sodium nitrite were 1:5 or 1:10 and 1:10, respectively. Reducing agents employed were ascorbic acid. erythorbic acid or ascorbyl palmitate. The reaction mixture was heated at 85 ± 1°C for 15 min with intermittent stirring upon which DNFH was formed. After cooling, the mixture was centrifuged for 15 min at 2000 rpm. The supernatant was separated and acidified to pH 4.0 with citric acid in order to precipitate out the resultant DNFH.

Yield and purity of DNFH, after exhaustive extraction, was determined. Pigments from a commercial sample of ham or DNFH.treated meat were extracted into 80% acetone#water as described by Hornsey (1956).

Colour Evaluation as Measured by Hunter L, a, b Values

Three types of pork muscles were used in this study. Type I was dark and beefish in appearance; Type III was fairly light and type II had an intermediate colour. These were obtained from Newfoundland Farm Products and were ground twice using a 0.79 cm and then a 0.48 cm plate.

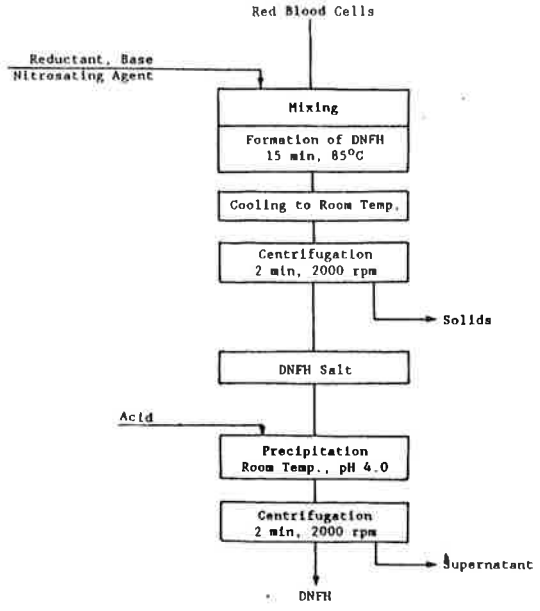

Figure 1. Flowsheet for direct preparation of dinitrosyl ferrohemochrom (DNFH) from bovine red blood cells.

Ground meats were mixed with 20% (w/w) of distilled water, 550 ppm sodium ascorbate and various additives (Table 1). The meat systems were cooked in a thermostatically controlled water bath at 85 ± 1°C for 45 min. After cooling to room temperature, cooked meats were homogenized in a Waring blender and transferred to Pyrex petri dishes for colour evaluation. The colorimeter was standardized with a white ceramic tile with the following specifications: L, 92.0; a, -1.1; and b, 0.7. An average value of five L, a, b readings was calculated for each sample. For each type of meat two control samples, one with no additives and the other cured with 156 ppm of sodium nitrite were used for colour comparison.

RESULTS AND DISCUSSION

Figure 1 illustrates the unit operations involved in the preparation of dinitrosyl ferrohemochrome, DNFH, from bovine red blood cells and sodium nitrite, in the presence of a reductant and in buffered solutions at elevated temperatures. Reducing agents employed kept the iron atom, in the iron-porphyrin complex, in its ferrous oxidation state and also hastened the conversion of nitrite to nitric oxide.

Effect of type and concentration of reductants, individually or in combination, on the yield and purity of DNFH are summarized in Table 1. While ascorbic and erythorbic acids were equally effective at a concentration equivalent to 10 times higher than that of the available hemin, they were ineffective at lower concentrations. Ascorbyl palmitate, however, was effective at a lower concentration (Table 1. Experiment No. 4). but gave lower yields of DNFH. The resultant pigment from the latter experiments was somewhat better in colour impartation to meats.

Variation of the concentration of sodium hydroxide in the preparation of DNFH showed that while its presence was

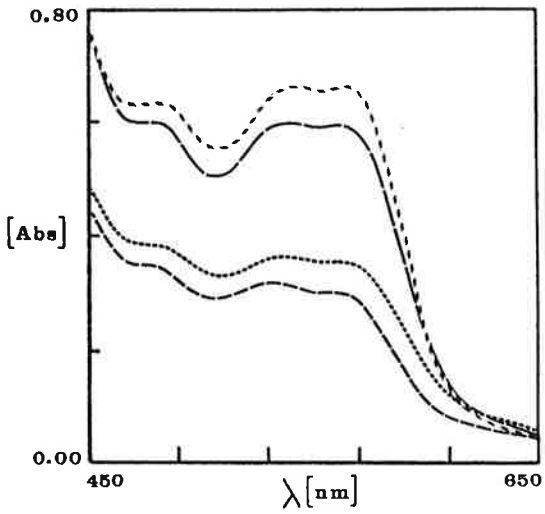

Figure 2. Absorption spectra of: DNFH prepared from red blood cells ------; DNFH prepared from hemin ————; pigments extracted from ham ---------- ; and pigments extracted from DNFH-treated cooked meat — — — —.

necessary for yield improvements, it did not have any considerable influence on the purity of the resultant pigment (Table 1).

Typical absorption spectra of DNFH prepared directly from bovine red blood cells or from hemin are shown in Figure 2. These are similar to the spectra of the pigments extracted from a commercial sample of ham or from the DNFH.treated meat. For all spectra identical absorption maxima were observed (Figure 2).

Table 2 summarizes the Hunter colour parameters L, a, b of ground pork (types I, II and III) cooked with water, sodium ascorbate and a variety of common food additives. For meats cooked with no additives, the level of myoglobin present in the samples influenced their Hunter L, a, b values. As expected, a decrease in the content of myoglobin in meats results in an increase in the values of L (or lightness) and a decrease in a (or redness) values.

Curing of meat (types I, II, and III) with 156 ppm of sodium nitrite brought about an increase in the a values. This is in agreement with the fact that curing develops a bright pink colour in meats. Similar results were obtained when DNFH (12 ppm) was used as a nitrite alternative for colour development. Addition of sodium tripolyphosphate (STPP) and sodium acid pyrophosphate (SAPP) to the DNFH.treated samples had a beneficial effect and further increased the a values.

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