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Colour Characteristics of Cooked Cured-Meat Pigment and its Application to Meat

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ABSTRACT

The colour characteristics of comminuted pork treated with pre-formed cooked cured-meat pigment and protoporphyrin-IX (PP-IX) were compared to that of nitrite-cured meat. Hunter L, a, b *values indicated that pigment-treated meat, at 12 ppm level, upon cooking, closely resembled that of nitrite-cured meat. Addition of sodium tripolyphosphate (STPP) and/or sodium acid pyrophosphate (SAPP) did not have a significant (*$P < 0.05$*) effect on the colour of pigment-treated meats as measured by Hunter* L, a, b *values. However, visually, STPP and, more notably, SAPP addition resulted in a more attractive product appearance. Meat samples treated with PP-IX, at all levels tested, upon cooking, resembled that of uncured meat as determined by Hunter* L, a, b *values.*

INTRODUCTION

Colour is an important sensory property of food and contributes greatly to its overall acceptability. The characteristic pink colour of cured-meats has a special role in consumer acceptance as it is usually associated with the quality of the processed meats. The role of nitrite in imparting the characteristic colour to cured-meats is well-established (Fox, 1966).

Concern over the use of nitrite due to its possible reactions with amines and amino acids present in meat has been the subject of many investigations (Gray & Randall, 1979; Sen *et al.*, 1973). *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine, examples of such reaction products, have been detected at low levels, < 100 ppb, in bacon after frying (Gray, 1976; Sen *et al.*, 1977)

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and were shown to be carcinogenic and possibly mutagenic and teratogenic in experimental animals (Magee & Barnes, 1967). The National Academy of Sciences (1982) has recommended that exposure of *N*-nitroso compounds to humans from nitrite-treated foods be reduced and strategies for long-term research on alternatives to nitrite usage be developed. This is also in line with the stated policy of the US and the Canadian governments (Holley, 1981).

In a recent paper, Killday *et al.* (1988) have shown that the cooked cured-meat pigment exists as a five coordinate mononitrosyl ferrohemochrome. This is contrary to the view of a dinitrosylheme complex proposed by Lee and Cassens (1976) and Tarladgis (1962). However, dinitrosyl ferrohemochromes under a positive pressure of nitric oxide do exist (Wayland & Olsen, 1974). Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome (DNFH), from hemin, extracted from bovine red blood cells, and sodium nitrite or nitric oxide has already been reported (Shahidi *et al.*, 1984; 1985). During hemin-nitric oxide synthesis, the pre-formed pigment is stored under a positive pressure of nitric oxide and may exist as a dinitrosyl ferrohemochrome complex; upon removal from this environment, decomposition of this pigment to a mononitrosyl complex is uncertain. Application of this pre-formed pigment to meat, upon cooking, gave a colour which was visually indistinguishable from that imparted by nitrite to cooked meats. Although this pigment had low stability in solutions under light and in the presence of oxygen, under a positive pressure of nitric oxide it was fairly stable (Shahidi *et al.*, 1985). In a more recent publication, Smith & Burge (1987) used iron-free protoporphyrin-IX as a substitute for nitrite, assuming it was a more stable colorant for possible industrial application by the meat processors.

Addition of phosphates, particularly sodium tripolyphosphate (STPP), has been shown to improve the colour, flavour and tenderness of meats (Smith *et al.*, 1984). Savich and Jansen (1954) reported that the colour of comminuted meat was stabilized for extended periods when phosphates were added.

The purpose of this study was to compare the colour of meats cooked with the pre-formed cooked cured-meat pigment, protoporphyrin-IX, and sodium nitrite, using Hunter *L*, *a*, *b* colour parameters. Furthermore, the effect of addition of sodium tripolyphosphate (STPP) and sodium acid pyrophosphate (SAPP), individually or in combination, on the colour characteristics of pigment-treated meats was investigated.

MATERIALS AND METHODS

All chemicals and solvents used in this study were reagent or food-grade. Protoporphyrin-IX (PP-IX) and hemin were purchased from the Sigma

Chemical Company, St. Louis, Missouri. The cooked cured-meat pigment was prepared from hemin and nitric oxide (Shahidi *et al.*, 1985).

Fresh pork loin, obtained from Newfoundland Farm Products, was trimmed of surface fat and ground twice with a 0·79 cm and then with a 0·48 cm plate. Ground pork was mixed with 20% by weight of distilled water and 550 ppm sodium ascorbate. Sodium nitrite, preformed cooked cured-meat pigment, PP-IX, STPP and SAPP were added directly to meat samples at various levels and as indicated in Tables 1–3. The mixtures were then thoroughly homogenized. Meat systems were cooked at 85 ± 2°C in a thermostated water bath for *ca.* 45 min to reach an internal temperature of 75 ± 2°C while stirring occasionally with a glass rod. After cooling to room temperature, cooked meat samples were homogenized in a Waring blender for 30 s and were then stored in the dark and at refrigeration temperature of 4°C in vacuum-packaged polyethylene pouches.

A colorimeter (Model XL-20 Tristimulus Colorimeter, Gardner Laboratory Inc., Bethesda, MD) was used to determine lightness (L value), red/green (+/− a value), and yellow/blue (+/− b value) of treated meats. A white ceramic tile with specifications $L = 92{\cdot}0$, $a = -1{\cdot}1$, and $b = 0{\cdot}7$ was used to standardize the colorimeter. The Hunter L, a, b colour values were measured at 3–5 different locations on the meat surface.

The total colour difference (ΔE) of treated meat samples, expressed as:

$$\Delta E = [(L - L_{ref})^2 + (a - a_{ref})^2 + (b - b_{ref})^2]^{1/2}$$

was calculated using 156 ppm nitrite-treated meat as reference.

TABLE 1
Hunter L, a, b Values of Treated Cooked Ground Pork[a]

Experiment Number	*Treatment*	*Hunter values*[b]			ΔE
		L	a	b	
1	No additives	59·1 ± 0·2*a*	5·6 ± 0·1*a*	11·8 ± 0·1*a*	8·4 ± 0·3
2	$NaNO_2$, 25 ppm	58·7 ± 0·1*a*	12·4 ± 0·1*b*	8·7 ± 0·2*b*	1·0 ± 0·3
3	$NaNO_2$, 50 ppm	58·5 ± 0·1*ab*	12·7 ± 0·1*bc*	8·7 ± 0·2*b*	0·7 ± 0·3
4	$NaNO_2$, 156 ppm	58·2 ± 0·4*abc*	13·3 ± 0·3*d*	8·6 ± 0·2*b*	Control
5	Pigment, 12 ppm	57·4 ± 0·6*bcd*	13·2 ± 0·1*cd*	8·7 ± 0·1*b*	0·8 ± 0·7
6	Pigment, 18 ppm	57·1 ± 0·7*cd*	13·5 ± 0·3*de*	8·4 ± 0·2*b*	1·1 ± 0·8
7	Pigment, 24 ppm	56·5 ± 0·4*d*	13·8 ± 0·1*e*	8·4 ± 0·1*b*	1·7 ± 0·6
8	PP-IX, 60 ppm	52·1 ± 0·4*e*	6·8 ± 0·2*f*	9·4 ± 0·2*c*	9·0 ± 0·5
9	PP-IX, 100 ppm	49·1 ± 0·1*f*	7·1 ± 0·1*fg*	9·3 ± 0·1*c*	11·0 ± 0·4
10	PP-IX, 150 ppm	46·2 ± 0·6*g*	7·6 ± 0·2*gh*	7·6 ± 0·1*d*	13·3 ± 0·7
11	PP-IX, 250 ppm	43·2 ± 0·1*h*	7·8 ± 0·2*h*	6·2 ± 0·1*e*	16·2 ± 0·4

[a] All samples were prepared with 20% distilled water, and 550 ppm sodium ascorbate. PP-IX, protoporphyrin-IX.
[b] Means followed by the same letters in a column are not significantly different ($P > 0{\cdot}05$) from one another as determined by Tukey's test.

TABLE 2
Effect of Polyphosphates on Hunter *L*, *a*, *b* Values of Pigment-Treated Comminuted Pork[a]

Experiment number	Treatment	Hunter Values[b]			ΔE
		L	*a*	*b*	
1	STPP (0·3%)[c]	58·3 ± 0·4*a*	13·4 ± 0·2*a*	8·6 ± 0·4*a*	0·2 ± 0·5
2	SAPP (0·3%)[c]	57·2 ± 0·6*bc*	13·9 ± 0·1*abc*	8·3 ± 0·1*a*	1·2 ± 0·6
3	STPP (0·3%)[d]	58·0 ± 0·3*ab*	13·9 ± 0·3*abc*	8·5 ± 0·2*a*	0·6 ± 0·4
4	SAPP (0·3%)[d]	57·0 ± 0·2*bc*	14·4 ± 0·2*c*	8·2 ± 0·1*a*	1·7 ± 0·4
5	STPP (0·15%) + SAPP (0·15%)[c]	57·9 ± 0·2*abc*	13·6 ± 0·2*ab*	8·4 ± 0·1*a*	0·5 ± 0·4
6	STPP (0·15%) + SAPP (0·15%)[d]	56·9 ± 0·4*c*	14·0 ± 0·2*ac*	8·2 ± 0·2*a*	1·5 ± 0·5

[a] All samples were prepared with 20% distilled water, and 550 ppm sodium ascorbate. STPP, sodium tripolyphosphate; SAPP, sodium acid pyrophosphate.
[b] Means followed by the same letters in a column are not significantly different ($P > 0{\cdot}05$) from one another as determined by Tukey's test.
[c] Pigment was added at 12 ppm level.
[d] Pigment was added at 24 ppm level.

Analysis of variance and Tukey's Studentized Range Test (Snedecor & Cochran, 1980) were used to determine differences in mean Hunter *L*, *a*, *b* values based on data collected from three replications of each treatment. Significance was determined at the 95% level of probability.

Absorption spectra of the pre-formed pigment and PP-IX dissolved in 4:1 (v/v) acetone:water solutions, as described by Hornsey (1956), were recorded using a Shimadzu UV-260 spectrophotometer. Meat pigments from the nitrite-, pre-formed pigment-, and PP-IX-treated pork, after cooking, were extracted into 4:1 (v/v) acetone:water. Allowance was made for moisture

TABLE 3
Effect of Polyphosphates on Hunter *L*, *a*, *b* Values of Nitrite-Cured Comminuted Pork[a]

Experiment number	Treatment	Hunter values[b]			ΔE
		L	*a*	*b*	
1	$NaNO_2$, 156 ppm	57·4 ± 0·2*a*	13·5 ± 0·2*a*	9·4 ± 0·1*a*	Control
2	(1) + SAPP (0·3%)	57·7 ± 0·2*a*	13·4 ± 0·2*a*	9·3 ± 0·1*a*	0·3 ± 0·3
3	(1) + SAPP (0·3%)	57·5 ± 0·3*a*	13·7 ± 0·1*a*	9·3 ± 0·1*a*	0·3 ± 0·2
4	(1) + STPP (0·15%) + SAPP (0·15%)	57·5 ± 0·2*a*	13·6 ± 0·2*a*	9·2 ± 0·1*a*	0·3 ± 0·2

[a] All samples were prepared with 20% distilled water, and 550 ppm sodium ascorbate. STPP, sodium tripolyphosphate; SAPP, sodium acid pyro-phosphate.
[b] Means followed by the same letters in a column are not significantly different ($P > 0{\cdot}05$) from one another as determined by Tukey's test.

content of the meat (Hornsey, 1956). All absorption spectra in the visible range were recorded.

RESULTS AND DISCUSSION

The Hunter *L*, *a*, *b* colour values of cooked ground pork treated with nitrite, pre-formed pigment and PP-IX are shown in Table 1. Addition of nitrite to fresh meat oxidized heme iron to the ferric state and produced a brown colour as expected and, upon cooking, the bright pink colour of cured-meat pigment was produced. The Hunter *L* and *b* values (a measure of lightness and yellowness, respectively) of cured meats at nitrite addition levels of 25–156 ppm were not significantly ($P < 0{\cdot}05$) different from one another. However, a significant ($P < 0{\cdot}05$) increase in the Hunter *a* value (a measure of redness) was evident at the 156 ppm nitrite addition level.

Addition of the pre-formed cooked cured-meat pigment to comminuted meat at 12, 18 and 24 ppm levels produced, upon cooking, a pink colour which was visually indistinguishable from that of nitrite-treated samples as judged by the authors. Hunter *L*. *a*, *b* values of pigment-treated samples at 12 and 18 ppm addition levels were not statistically different ($P < 0{\cdot}05$) from that of 156 ppm nitrite-cured control. The meat sample treated with 24 ppm pigment was darker (significantly ($P < 0{\cdot}05$) lower *L* value) and more pinkish (significantly ($P < 0{\cdot}05$) higher *a* value) compared to that of the nitrite control. Although greater pigment concentrations resulted in a darker product, as indicated by decreasing *L* values (Table 1), an analysis of variance test showed that the recorded mean values of the three pigment levels examined were not significantly ($P < 0{\cdot}05$) different from one another. Hunter *a* values increased significantly ($P < 0{\cdot}05$) as the pigment addition level was increased from 12 to 24 ppm. No significant ($P < 0{\cdot}05$) effect on Hunter *b* values was noted at all pigment levels tested.

Addition of protoporphyrin-IX (PP-IX), to fresh meat at 60, 100, 150 and 250 ppm levels, produced a purple-red colour. Upon cooking, PP-IX-treated meats turned dark brown. Although Hunter *a* values increased significantly ($P < 0{\cdot}05$) with added PP-IX, however, the treated samples were visually similar to that of the control with no additives. Furthermore, meats treated with increasing levels of PP-IX became significantly ($P < 0{\cdot}05$) darker as indicated by decreasing *L* values (Table 1) and were visually unappealing as judged by the authors. Use of PP-IX in comminuted meat systems did not mimic the pink colour imparted by nitrite or the pre-formed cooked cured-meat pigment upon heat processing. As expected, the absence of iron in the conjugated PP-IX precluded the development of the characteristic cured-meat colour in the final products (Giddings, 1977).

The overall colour difference (ΔE) of treated-cooked meats was measured. The sample cured with 156 ppm $NaNO_2$ was used as the control. The range of ΔE between untreated and nitrite-cured meat samples was $8{\cdot}4 \pm 0{\cdot}3$. Colour difference values of all nitrite- and pigment-treated meats, with the exception of pigment added at 24 ppm level, were not significantly ($P < 0{\cdot}05$) different. Even though the ΔE for the pigment-treated sample at 24 ppm was significantly ($P < 0{\cdot}05$) different from that of the cured control, the visual colour of the treated sample was quite appealing as noted by the authors. Protoporphyrin-IX-treated meats had ΔE values of $\geq 9{\cdot}0$. A statistical difference ($P < 0{\cdot}05$) in ΔE values, compared to that of the uncured sample, was noted for all levels of PP-IX tested, with the exception of 60 ppm level of PP-IX addition.

The effect of polyphosphates on Hunter *L*, *a*, *b* values of pigment- and nitrite-cured comminuted pork is presented in Tables 2 and 3, respectively.

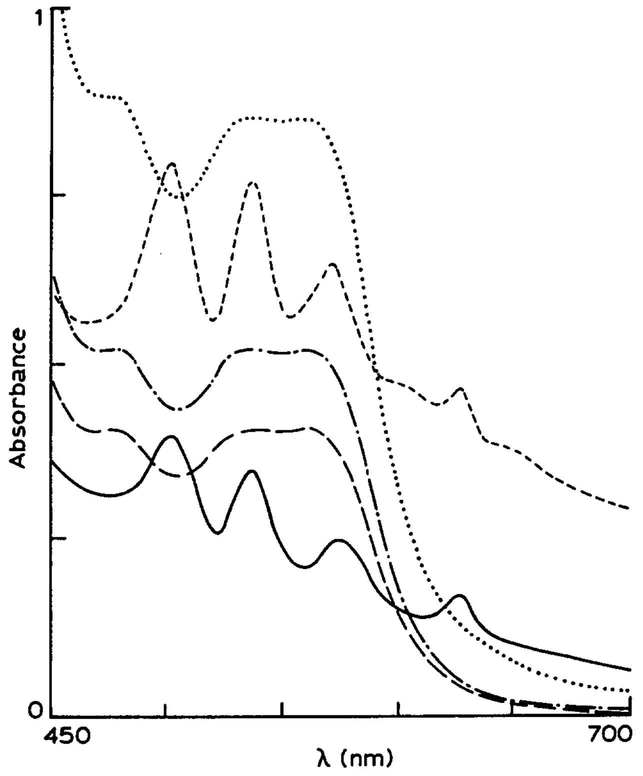

Fig. 1. Absorption spectra: ······, pre-formed pigment in 4:1 (v/v) acetone: water; -----, PP-IX in 4:1 (v/v) acetone:water; —·—·—, pigments extracted from pigment-treated cooked meat; ——, pigments extracted from nitrite cured meat; ———, pigments extracted from PP-IX-treated cooked meat.

Addition of sodium tripolyphosphate (STPP) and sodium acid pyrophosphate (SAPP), individually or in combination, to pigment and nitrite-treated meats did not have a significant ($P < 0.05$) effect on colour of the resultant products. Although no significant ($P < 0.05$) effect was noted from the objective method of analysis, pigment-treated samples with added STPP and SAPP were visually more attractive in appearance as observed by the experimenters. Sodium acid pyrophosphate usage seemed to improve the colour characteristics of pigment-treated meat to a greater extent than that of STPP at an equal addition level. Furthermore, the mechanism by which STPP and SAPP affect colour enhancement in pigment-treated meats remains unknown.

The absorption spectrum of the pre-formed cooked cured-meat pigment in 4:1 (v/v) acetone:water solution was identical to the pigments extracted from nitrite- and pigment-treated meats, after cooking (Fig. 1). Similar maxima at 540 and 563 nm were noted for the pigment extracts in all cases. The spectrum of PP-IX was similar to the spectrum of pigments extracted from PP-IX-treated meats, after cooking.

The colour characteristics of comminuted meats treated with 12 ppm of the pre-formed cooked cured-meat pigment were similar to that of nitrite-cured meat, at 156 ppm addition level. Although no nitrite was added to systems containing the cooked cured-meat pigment, transnitrosation of the heme compounds present in meat with the nitric oxide moiety(ies) of the cooked cured-meat pigment may occur. Thus, the pre-formed cooked cured-meat pigment seems to be a viable alternative for imparting colour in non-nitrite curing of comminuted meat products.

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REFERENCES

Fox, J. B., Jr (1966). The chemistry of meat pigments. *J. Agric. Food Chem.*, **14**, 207.

Giddings, G. C. (1977). The basis of color in muscle foods. *CRC Crit Rev. Food Sci. Nutr.*, **4**, 81.

Gray, J. I. (1976). *N*-Nitrosamines and their precursors in bacon: A review. *J. Milk Food Technol.*, **39**, 686.

Gray, J. I. & Randall, C. J. (1979). The nitrite/*N*-nitrosamine problem in meats: An update. *J. Food Prot.*, **42**, 168.

Holley, R. A. (1981). Review of the potential hazard from botulism in cured meats. *Can. Inst. Food Sci. Technol. J.*, **14**, 183.
Hornsey, H. C. (1956). The colour of cooked cured pork. I. Estimation of the nitric oxide-haem pigments. *J. Sci. Food Agric.*, **7**, 534.
Killday, K. B., Tempesta, M. S., Bailey, M. E. & Metral, C. J. (1988). Structural characterization of nitrosylhemochromogen of cooked cured meat: Implications in the meat-curing reaction. *J. Agric. Food Chem.*, **36**, 909.
Lee, S. H. & Cassens, R. G. (1976). Nitrite binding sites on myoglobin. *J. Food Sci.*, **41**, 969.
Magee, P. N. & Barnes, J. M. (1967). Carcinogenic nitroso compounds. *Adv. Cancer Res.*, **10**, 163.
National Academy of Sciences. (1982). Alternatives to the Current Use of Nitrite in Foods. National Academy of Science, Washington, DC.
Savich, A. L. & Jansen, C. E. (1954). Process for improving the color of animal material. US Patent 2 830 907.
Sen, N. P., Miles, W. F., Donaldson, B., Panalaks, T. & Iyengar, J. R. (1973). Formation of nitrosamines in a meat curing mixture. *Nat.*, **245**, 104.
Sen, N. P., Donaldson, B., Seaman, S., Collins, B. & Iyengar, J. R. (1977). Recent nitrosamine analyses in cooked bacon. *Can. Inst. Food Sci. Technol. J.*, **10**, A13.
Shahidi, F., Rubin, L. J., Diosady, L. L., Chew, V. & Wood, D. F. (1984). Preparation of dinitrosyl ferrohemochrome from hemin and sodium nitrite. *Can. Inst. Food Sci. Technol. J.*, **17**, 33.
Shahidi, F., Rubin, L. J., Diosady, L. L. & Wood, D. F. (1985). Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. *J. Food Sci.*, **50**, 272.
Smith, J. S. & Burge, D. L., Jr (1987). Protoporphyrin-IX as a substitute for nitrite in cured-meat color production. *J. Food Sci.*, **52**, 1728.
Smith, L. A., Simmons, S. L., McKeith, F. J., Bechtel, P. I. & Brady, P. L. (1984). A research note. Effects of sodium tripolyphosphate on physical and sensory properties of beef and pork roasts. *J. Food Sci.*, **49**, 1636.
Snedecor, G. W. & Cochran, W. G. (1980). *Statistical Methods*, (7th edn). The Iowa State University Press, Ames, IA.
Tarladgis, B. G. (1962). Interpretation of the spectra of meat pigments. II. Cured meats. The mechanism of colour fading. *J. Sci. Food Agric.*, **13**, 485.
Wayland, B. B. & Olsen, L. W. (1974). Spectroscopic studies and bonding model for nitric oxide complexes of iron porphyrins. *J. Am. Chem. Soc.*, **96**, 6037.