The Stabilization of Dinitrosyl Ferrohemochrome and its Application in the Production of Nitrite-Free Pork Cuts

A.R. O'BOYLE, N. ALADIN-KASSAM, L.J. RUBIN and L.L. DIOSADY
Department of Chemical Engineering, University of Toronto, Toronto, Canada, M5S 1A4

SUMMARY: The preformed cured-meat pigment undergoes rapid oxidation in the presence of air and light. It can be stabilized by microencapsulation using spray drying. An essential ingredient of the encapsulating material is β-cyclodextrin. The rest consists of modified starches such as N-Lok or maltodextrin. The encapsulated pigment remained stable for over one year. It could be readily used in the production of nitrite-free wieners. In the case of solid meat cuts such as ham, the even distribution of the sparingly soluble pigment proved to be difficult. This problem was overcome by careful control of particle size of the pigment, by multiple injection of pickle with a modified needle, by tumbling both before and after injection, and by cooking in sealed bags in a water bath.

INTRODUCTION: One of the key functions of nitrite is to impart to meat the characteristic cured-meat colour. In addition, it prevents lipid oxidation bringing forth the characteristic cured-meat flavour (RUBIN & SHAHIDI, 1988), and inhibits the outgrowth of and toxin formation by food poisoning microorganisms, especially C. botulinum. However, the use of nitrite as a food additive is subject to serious concern. It can form carcinogenic nitrosamines in food (SCANLAN, 1975) and possibly also in the stomach (WISHNOK, 1977). It therefore seems prudent to search for a replacement for nitrite.

It has long been realized that the chance of finding a single compound which duplicates all the functions of nitrite is very minute. However, a multicomponent system, including the synthetic cooked cured-meat pigment DNFH, could produce a nitrite-free cured-meat product identical in all the key aspects -- colour, oxidative stability and flavour, and preservative effect -- to a nitrite-cured product. This has been the focus of our research for a number of years. The system is described in a patent (RUBIN et al., 1985) and in a recent paper which deals with the production of nitrite-free wieners (O'BOYLE et al., 1990).

The cooked cured-meat pigment, dinitrosyl ferrohemochrome (DNFH), is prepared chemically from hemoglobin (SHAHIDI et al., 19[illegible] SHAHIDI et al., 1985). The basic starting material for this synthesis is beef red blood cells, from which the iron (III) porphyrin hemin is prepared. The pigment is then produced by treating a buffered solution of hemin in the presence of a reducing agent with nitric oxide. The pigment is recovered as a fine crystalline material which is blackish-red in colour and sparingly soluble in water. Recently, KILLDAY et al. (1988) have suggested that the cooked cured-meat pigment is a mononitrosyl rather than a dinitrosyl species. This question remains unresolved.

Another major problem with synthetically prepared DNFH is that, like the pigment of cooked cured meat, it is very susceptible to light-induced oxidation. This property leads to colour fading in nitrite-cured meat, and also means that the synthetic pigment cannot be readily stored in its native form. One obvious approach to protecting the pigment is to use the technique of microencapsulation.

Microencapsulation is defined as the technology of packaging minute quantities of solid, liquid, or gas within continuous, individual walls. These walls are designed to release their contents in a predictable manner, under a predetermined set of conditions (TODD, 1970). Microencapsulation has found many applications in chemical processing and, in particular, in the food industry. It is commonly used to coat and protect sensitive ingredients such as aromas, flavours, and vitamins. The most commonly used method of microencapsulation in the food industry is spray drying (DZIEZAK, 1988), because it is economical, flexible, and continuous. The process of spray drying itself is discussed very thoroughly by MASTERS (1985).

Recently, a great deal has been written about the unique encapsulating ability of a cyclic glucose polymer known as β-cyclodextrin (LINDNER et al., 1981; SZEJTLI, 1982; ANON., 1988). This torus shaped molecule can encapsulate molecules (or functional groups of molecules) at the molecular level. This process is referred to as "inclusion complexation" and allows, among other things, the stabilization of light- or oxygen-sensitive materials. Accordingly, the encapsulation of the preformed cooked cured-meat pigment in a series of natural polymers, including β-cyclodextrin, has been studied.

Unlike nitrite, the pigment DNFH is a very large (M.W. 676.5) molecule which is sparingly soluble in water or curing pickle, and thus does not readily diffuse through an intact matrix of muscle fibres. It is believed that the collagenous coating around bundles of muscle fibres (perimysium) offers the major resistance to the transport of DNFH. The relative immobility of the pigment is an obvious stumbling block in the production of the nitrite-free hams.

MATERIALS AND METHODS: The cooked cured-meat pigment DNFH was prepared using commercially produced bovine crystalline hemin (Aldrich Chemical Co. Inc., Milwaukee, WI) by the method of SHAHIDI et al. (1985), with some modifications.

The carbohydrate wall materials used to encapsulate the pigment were food-grade. They were used along, or in combination, and included: β-cyclodextrin (Sanraku Inc., Tokyo, Japan, and American Maize-Products Co., Hammond, IN); N-Lok, a modified starch (National Starch and Chemical Corp., Bridgewater, NJ), maltodextrin with D.E. of 25.0 (Maltrin M-250, Grain Processing Corp., Muscatine, IA).

The curing pickle was prepared using distilled water, salt (BDH Chemicals, Toronto, Canada), sucrose (commercial brands), sodium tripolyphosphate (Erco Industries Ltd., Islington, Canada), sodium ascorbate (Sigma Chemicals, St. Louis, MO), and sodium hypophosphite

(BDH Chemicals, Toronto, Canada), all of which were food-grade. The meat was obtained from Canada Packers Inc. (Toronto, Canada), in the form of whole, uncured, bone-in hams.

Encapsulation of the Pigment: An appropriate amount of encapsulating agent was weighed, keeping in mind the desired capsule payload and feed solids content. For example, an encapsulated pigment with a desired loading of 2% and a feed solids content of 10% would be prepared by coating 0.2 g of DNFH with 10 g of wall material in a feed dispersion whose volume was approximately 100 mL. Sodium ascorbate was added to the feed dispersion as an antioxidant in an amount such that the ratio (w/w) of ascorbate to DNFH was approximately 0.5. As a first step, the antioxidant and wall material were dissolved in distilled water to form the continuous phase of the feed dispersion. A small amount (approx. 0.5 mL per 100 mL) of 50% (w/w) sodium hydroxide was added, bringing the pH of the solution up to approximately 11 to aid in the dissolution of β-cyclodextrin.

In the meantime, the DNFH was separated from the buffered solution in which it was prepared (by centrifugation), and then dispersed in a small quantity (25-50 mL) of distilled water. This dispersion was treated, under nitrogen, with a Polytron Homogenizer (Brinkman Instruments, Model PT 10/35, PTA-20S generator) for 2 min at an instrument setting of '6' (10,000 RPM). The purpose of this process was to reduce the average size of the pigment particles. This "homogenized" DNFH (disperse phase) was added to the aforementioned solution of wall material (continuous phase) and the entire feed dispersion was further treated with the Polytron (setting of '5', ~ 1 min) to ensure feed homogeneity prior to spray drying.

The Büchi 190 mini spray dryer, which was used to encapsulate the DNFH, was equipped with a pneumatic nozzle atomizer and operated on the principle of co-current flow of drying air and product spray. Details of this procedure will be given elsewhere.

The pigment could not be readily extracted from the microencapsules with 80% acetone for analysis using the method of HORNSEY (1956). It was, therefore, judged by its ability to impart a pink colour to ground pork as compared to a 150-ppm nitrite control. Lean ground pork (40 g) was weighed into a 150 mL beaker. Spray-dried pigment, usually 30-50 ppm DNFH, and 550 ppm of sodium ascorbate (both based on 50 g total weight of meat and water), were dispersed in 10 mL of distilled water. This dispersion was then added to the ground pork and a glass rod was used to thoroughly mix the pigment into the pork. The meat sample was cooked in a constant temperature bath for approximately 40 min at 85°C. The colour of the cooked sample was then judged against that of the nitrite control. A rating of 10 was given to the nitrite-cured control while a rating of 1 implied no colour present (i.e., same as cooked, uncured ground pork).

Representative particle-size distributions of the pigment were obtained using a Coulter Counter with an aperture diameter of 70 microns.

Preparation of Nitrite-Free Hams: Meat cuts (i.e., the inside, outside, and round muscles of the ham) were obtained by manual deboning. Individual whole muscles were used for each experimental run in order to remove the inherent variability that different muscle groups would present.

The aqueous curing pickle was prepared on the basis of a 20-30% gain in the fresh weight of the meat. The concentrations of the various ingredients in the pickle were such as to produce approximately the following levels in the final cured product (before cooking): salt, 2%; sucrose, 1%, ascorbate, 0.06%; sodium tripolyphosphate (STPP), 0.3%; sodium hypophosphite, 0.3%; and dinitrosyl ferrohemochrome, 30-40 ppm (mg/kg). Sodium hypophosphite was used as an antimicrobial agent (WOOD et al., 1986).

The DNFH was combined with the rest of the curing brine immediately before injection into the meat. It was imperative that the DNFH be finely ground and well-dispersed in the injection solution. With this in mind, the entire DNFH/pickle mixture was treated with the Polytron homogenizer prior to injection. Such a step was indeed critical in obtaining uniform distributions of pigment within the solid cuts of ham. The treatment consisted of 1-2 min of homogenization, under nitrogen, at an instrument setting of '6' (10,000 RPM).

The resulting "homogenized" pickle dispersion was injected into the meat tissue. A multiple-injection technique was used to ensure that each section of the cut received an equal volume of pickle. The injection needle (1.5 mm diameter) was made with a series of 4 holes along its length to allow pickle to radiate outwards from the entire length of the needle and the tip was blocked to prevent downward flow. The needle was small enough to allow for a great many injection sites in each cut. The sites were spaced evenly at a distance of 1-2 cm.

After injection, the meat was physically treated by tumbling in order to accelerate the curing process and to hasten the movement of the pigment. The tumbler was located in a refrigerated room and the runs were performed at 0-1°C. The duration of the tumbling was usually 9 h. Normally, the ratio of tumbling time to relaxation time was 15 min to 45 min. The rotational speed of the tumbler was 10-15 rpm. A 3 h (continuous) tumbling treatment of the fresh meat, prior to pickle injection, was also tested for its effect on pigment distribution.

After the physical treatment, the cured meat sample was sealed in a freezer bag and stored in a refrigerator for two days prior to cooking. The meat was then cooked in a water bath (at 75-80°C) to an internal temperature of 65-70°C. Finally, the meat was sliced (in many directions) and inspected for colour quality and uniformity.

RESULTS AND DISCUSSION:

Microencapsulation of DNFH: The DNFH content, or payload, of encapsulated pigment was varied from 1 to 4%. At the higher payloads, encapsulation was incomplete. A 2% payload seemed like a reasonable compromise. Table 1 shows some of the encapsulating combinations tested and the corresponding experimental results. Only the combinations of β-cyclodextrin with N-Lok and Maltrin M-250 will be shown here. A fuller report will appear elsewhere.

Table 1. Encapsulating Agents and DNFH Quality[a]

β-CD[b] (%)	N-LOk (%)	Maltrin (%) M-250	Colour Quality[c]
100			9.5
90	10		9.5
80	20		9.5
70	30		10.0
60	40		10.0
50	50		9.5
40	60		10.0
30	70		8.5
20	80		10.0
10	90		9.5
	100		5.5
90		10	9.0
80		20	9.5
70		30	10.0
60		40	10.0
50		50	9.5
40		60	10.0
30		70	10.0
20		80	10.0
10		90	9.5
		100	5.5

[a]Products were examined within one week of being prepared. The payload was 2%.
[b]β-cyclodextrin.
[c]Colour Quality: 10 - colour of nitrite control; 1 - colour of cooked uncured pork; 1-5, bad; 6-7.5, acceptable; 8-9, very good; 10 - excellent.

The results indicate clearly that β-cyclodextrin is the essential ingredient of the encapsulating mixture. It works well by itself but it need only be present at a level of 10[illegible] when mixed with N-Lok or Maltrin M-250. Similar results were obtained with gum arab[illegible] and Maltrin 040, but these are not reported here. When no β-cyclodextrin is used, there[illegible] sharp drop in pigment quality. The fact that the level of β-cyclodextrin can be lowered to[illegible] or 20% is important, since it is the expensive ingredient in the encapsulating mixture.

Clearly, β-cyclodextrin has unique properties which influence pigment stability. It[illegible] nevertheless unlikely that the encapsulation of DNFH takes place at the molecular level s[illegible] the molecule is too large to fit into the 7.8 angstrom cavity of β-cyclodextrin. However,[illegible] possibility does exist that certain functional groups of DNFH, particularly the axial nitric[illegible] oxide ligands, could be included. Thus, the nitric oxide groups could be protected again[illegible] dissociation and subsequent oxidation. It is also conceivable that DNFH molecules are h[illegible] between several cyclodextrin rings, by so-called "crystal lattice inclusion" (LINDNER et[illegible] 1981).

Encapsulated DNFH powder was stored, at room temperature and in the dark, in se[illegible] glass sample bottles. In general, it appeared that encapsulated DNFH which was of good[illegible] quality initially, stayed that way for quite some time. The encapsulated pigments for the[illegible] series shown in Table 1 were still satisfactory after 14 months of storage. The pigment[illegible] quality may have deteriorated somewhat, but the colour imparted to pork was still more[illegible] acceptable. The stability of the encapsulated pigment should meet commercial shelf-life[illegible] requirements.

Application of DNFH to the Production of Nitrite-Free Hams: The achievement of a[illegible] uniform distribution of pigment within the solid meat cut is a difficult task. The two main[illegible] reasons for this are the limited solubility of the DNFH and the complex structure of meat, as referred to earlier. Over 130 experimental runs[illegible] were done, and the result can only be summarized here. The encapsulated pigment was used successfully in the preparation of wieners where[illegible] fine comminution eliminated all problems of pigment distribution (O'BOYLE et al., 1990).

Given the fact that DNFH has difficulty in moving within the meat tissue, the manner in which it is injected becomes all-important. By[illegible] covering the meat surface with a great many evenly spaced injection points, one can, in essence, induce a uniform distribution of pigment to[illegible] make up for its limited mobility. The standard hypodermic syringe, having just a single orifice, allowed only unidirectional flow of pickle.[illegible] This, in part, caused unappealing vertical lines of high DNFH concentration within nitrite-free hams. By forcing the pickle solution to radiate[illegible] outwards in several directions, the multi-orifice needle provided random and complete penetration of DNFH into the meat tissue. Thus, the[illegible] multi-orifice needle was judged as the best of the available means of pickle injection.

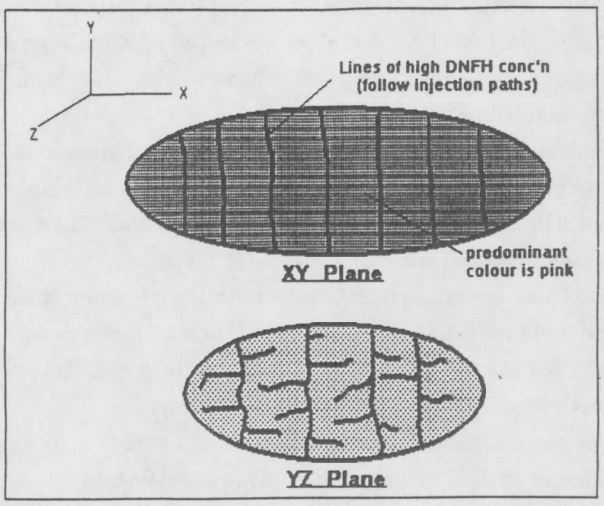

Figure 1. Typical pigment distribution pattern in ham injected with an ordinary hypodermic needle.

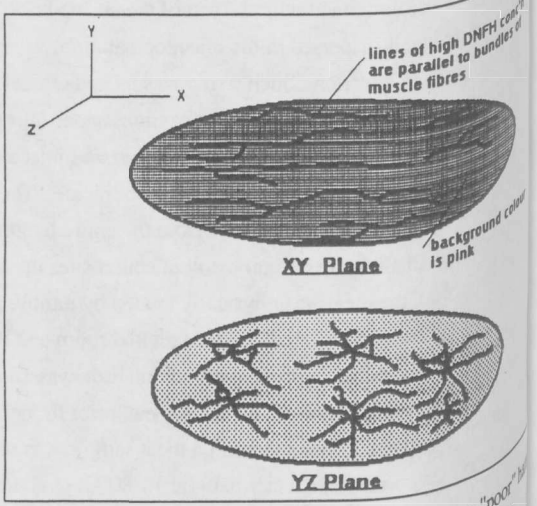

Figure 2. Typical pigment distribution pattern in a "poor" ham injected with multi-orifice hypodermic needle.

Figure 1 depicts, in a somewhat exaggerated manner, the pattern of vertical lines of high DNFH concentration which often appeared when the simple hypodermic needle was used for injection. These dark red lines followed the injection paths and, as such, were perpendicular to the bundles of muscle fibres. In this figure, as in the next, the muscle fibres are shown in lateral section in the XY plane and in cross-section in the YZ plane. Figure 2 illustrates the distribution of high-DNFH concentration zones in non-uniform ham products which were injected with

the multi-orifice needle. The lines were primarily along the seams between the bundles of muscle fibres. It was apparent that, although DNFH had difficulty moving in any direction within the muscle tissue, it moves more readily along the length of muscle fibres than perpendicular to them. The latter motion would require diffusion through protection sheaths of collagen which surround both the individual fibres and groups of fibres.

Particle size was considered to be the most important factor governing the diffusion of pigment through muscle tissue. An aqueous dispersion of DNFH (unencapsulated), which has been treated with the Polytron, had a median particle diameter of 2.2 microns as measured with the Coulter Counter. Encapsulated pigment dispersed in water and also treated with the Polytron had a median particle diameter of 2.6 microns. In contrast, a dispersion of raw, unencapsulated and untreated DNFH had a median particle size of 8 microns. The DNFH/pickle mixture was treated with the Polytron prior to injection. As an added precaution, this "homogenized" pickle was sometimes centrifuged to remove the coarser particles. The resulting hams were the most visually appealing to date. The interior colour was a strong bright pink, with no uncoloured areas.

Tumbling was used to facilitate the penetration of DNFH through the muscle tissue. An intermittent tumbling routine of 9 h duration, at a speed of 12 rpm, with an hourly tumbling time of 15 min and a relaxation time of 45 min, proved to be quite suitable for this purpose. More intensive treatment yielded no perceptible improvement in the distribution of DNFH.

In an attempt to minimize the inhibitory effect of the muscle internal structure on DNFH migration, a pre-injection tumbling treatment was used. This was intended to "open-up" the muscle interior through partial fibre breakdown and disruption of collagenous membranes. The usual pre-injection treatment consisted of 3 hours of continuous tumbling. It is believed that this process did indeed benefit the distribution of DNFH in the meat tissue.

The cooking procedure affected the distribution of DNFH mainly due to its effect on water retention. Cooking of the nitrite-free hams in sealed bags in a water bath proved to be superior to using a convection oven. There was a significant increase in moisture retention (i.e., a weight gain of about 10% as opposed to losses anywhere from 2 to 30% when the oven was used). This moisture retention seemed to have a positive effect on colour uniformity as the high DNFH-concentration zones were not nearly as prominent as in the oven-cooked samples.

CONCLUSIONS: The preformed microencapsulated DNFH, as one element of a multicomponent meat-curing system, makes possible the preparation of nitrite-free cured-meat products such as hams or wieners.

Ideally, one envisions a process in which a fine powder of DNFH is used within a narrow particle-size range (probably between 1 and 2 microns). Under these conditions, we believe that complete dispersion of DNFH within intact meat tissue can be achieved and hence, nitrite-free hams (and other similar cured-meat products) would become a reality.

REFERENCES:

ANONYMOUS (1988): Production and potential food applications of cyclodextrins. Food Technol. 42(1):96.

DZIEZAK, J.D. (1988): Microencapsulation and encapsulated ingredients. Food Technol. 42(4):136.

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. and METRAL, C.J. (1988): Structural characterization of nitrosylhemochromogen of cooked cured meat: Implications in the meat-curing reaction. J. Agric. Food Chem. 36:909.

LINDNER, K., SZENTE, L. and SZEJTLI, J. (1981): Food flavouring with β-cyclodextrin-complexed flavour substances. Acta Alimentaria 10(3):175.

MASTERS, K. (1985): "The Spray Drying Handbook". John Wiley and Sons, New York.

O'BOYLE, A.R., RUBIN, L.J., DIOSADY, L.L., ALADIN-KASSAM, N., COMER, F. AND BRIGHTWELL, W. (1990): The development of a nitrite-free meat-curing system and its application to the production of wieners. Food Technol. 44:88.

RUBIN, L.J., DIOSADY, L.L., SHAHIDI, F. and WOOD, D.F. (1985): Meat Curing Compositions and Method of Use. U.S. Patent No. 4,559,234.

RUBIN, L.J. and SHAHIDI, F. (1988): Lipid oxidation and the flavour of meat products. Proceedings, 34th International Congress of Meat Science and Technology, Brisbane, Australia, p. 295.

SCANLAN, R.A. (1975): N-nitrosamines in foods. CRC Crit. Rev. Food Technol. 5:357.

SHAHIDI, F., RUBIN, L.J., DIOSADY, L.L., CHEW, V. and WOOD, D.F. (1984): Preparation of dinitrosyl ferrohemochrome from hemin and sodium nitrite. Can. Inst. Food Sci. Technol. J. 17(1):33.

SHAHIDI, F., RUBIN, L.J., DIOSADY, L.L. and WOOD, D.F. (1985): Preparation of the cooked cured-meat pigment, dinitrosyl ferrohemochrome, from hemin and nitric oxide. J. Food Sci. 50:272.

SZEJTLI, J. (1982): "Cyclodextrins and their Inclusion Complexes". Akademei Kiado, Budapest.

TODD, R.D. (1970): Microencapsulation and the flavour industry. The Flavour Industry 1(11):768.

WISHNOK, J.S. (1977): Formation of nitrosamines in food and in the digestive system. J. Chem. Educ. 54:440.

WOOD, D.S., COLLINS-THOMPSON, D.L., USBORNE, W.R. and PICARD, B. (1986): An evaluation of the antibotulinal activity in nitrite-free curing systems containing dinitrosyl ferrohemochrome. J. Food Protection 49:691.