NITRITES AND NITROSAMINES IN PROCESSED MEATS

SESSION F: CHEMISTRY

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Four of the papers in this Session are directly concerned with nitrites, curing and nitrosamines, the other two relate to other aspects of meat composition and analysis. I propose to deal with them in an order which is different from that in which they are printed.

From the beginning of investigations of the nitrosamine question in cured meats one of the most puzzling questions has been to account for the nitrite which is converted into other substances after being added to the meat. By way of introduction let us consider the picture as it was described by Sebranek and his co-workers in the University of Wisconsin last year.(1)

Fig 1 summarises their findings in pork meat treated with ^{15}N labelled nitrite, heated to 71°C at the time of preparation and then stored in cans for several weeks at 5°C. The residual nitrite content of the meat fell in a predictable manner. The nitrite bound as pigment remained roughly constant, and the fall in residual nitrite was approximately balanced by an increase in water soluble nitrogen, which would of course include any nitrate formed from the nitrite, though this was not separately measured. A substantial proportion of the labelled nitrogen was associated with the non-water

soluble proteins and the proportion bound to salt-insoluble proteins increased on storage. A constant 5% appeared as gases, which have been shown to include nitrogen and nitrogen oxides[2]. Note the relatively high proportion still unaccounted for, which should lead to caution in interpreting all of the other values.

Paper F2: of this Session, by Goutefongea, Renerre and Valin describes an experiment to establish the proportion of the nitrite added to a cured meat system which becomes bound to the myofibrils, that is, to the salt-soluble protein. These workers separated myofibrils from the rest of the meat, using an established technique, treated them with salt and sodium nitrite and applied heat processes representative of commercial practice and similar to those used by Sebranek et al. They then precipitated the protein with zinc ferrocyanide and determined the nitrite remaining in the solution: the difference between this and the original amount added was considered to be bound to the protein. Their results show that about 10% of the added nitrite or 18 ppm as sodium nitrite was bound in this way. The proportion bound was slightly increased in the presence of salt but was unaffected by the heat treatment or by pH in the range studied, from pH 5.5 - 6.5. Sebranek et al found a somewhat smaller amount of nitrite bound to myofibrils, which Goutefongea and his colleagues consider is probably due to the use of whole meat by Sebranek's group compared with suspended myofibrils in this study. They indicate that they hope to extend the work and to confirm the American findings for the other fractions of whole meat.

Let us now turn to those contributions which study directly

the curing process in meat products, commencing with Paper F4 by the Russians Palmin, Prisenki, Fydorova and Loginova. This reports two interesting techniques for producing 'cured' meat products with zero residual nitrite content. In the first of these they used the sodium salt of hemin chloride, whose preparation was described in a paper read at our meeting in Paris a year ago[3]. When this salt was added to a boiled sausage cured with nitrite and ascorbate in the conventional way there appear to have been three associated effects.

1. The added hemin salt combined with residual nitrite and thus reduced the residual nitrite content. The molecular weights of sodium hemin chloride and sodium nitrite are almost exactly in the ratio 10 to 1, and it can be calculated from Table 1 that at low levels of added nitrite the two substances reacted in this same ratio. 0.5 mg of sodium hemin chloride reduced the residual nitrite level by approximately 0.05 mg.

2. At the same time there was an apparent increase in the pigment conversion ratio. However, their Table 1 shows that the increase was approximately constant for constant increments of added sodium hemin chloride, even when no residual nitrite remained to react with it. Presumably unreacted hemin chloride was extracted in the same way as nitrosyl pigments in the analysis. The nitrosyl pigment conversion ratios quoted may therefore not truly represent the redness of the samples.

3. Formation of diethyl nitrosamine and dimethyl nitrosamine was eliminated, as shown in their Table III, presumably as a consequence of elimination of residual nitrite.

The second technique reported by these authors consists of preparing a meat product without added nitrite and colouring it with an added pigment. In this case the pigment was nitrosyl hemochromogen, the natural pigment of cured meat but prepared separately from hemin chloride. It is demonstrated in their Table 11 that the optical density of acetone extracts of the meat product increased proportionally to the amount of added pigment, as one would expect. Their Table 111 shows, as one would expect, that in the absence of added nitrite no nitrosamines were formed.

The problem with both of these techniques, which became very clear in work in our own laboratory two or three years ago using nitric oxide to produce cured meats with zero nitrite,[4] is of course that although one may have very good colour and low nitrosamine formation, there may be severe microbiological penalties and possibly deficiencies in flavour, resulting from the absence of residual nitrite. The authors claim that products made by their methods meet the appropriate official quality standards in Russia but they do not give any evidence of shelf life, safety to public health or flavour by which such a claim might be evaluated.

The contribution by Wierbicki, Heiligman and Wassermann, of the US Army Natick Laboratory, Paper F6, approaches this question from the other direction. They are concerned with the postulate that one can obtain satisfactory organoleptic properties in a cured meat using concentrations of nitrite too low to secure adequate microbiological protection, but then reinforce the microbiological

position by applying mild irradiation. In this study they have not in fact made any microbiological tests but they have clearly shown that there were no detectable ill effects organoleptically, and that satisfactory smoked ham and bacon were made with ingoing nitrite concentrations of only 25 ppm. Even at this level, nitrosamines were formed in the bacon on frying, whether the bacon was irradiated or not. The main value of this procedure would therefore lie in its contribution to reducing the intake of dietary nitrite. Although the authors' experiments were conducted on ham and bacon made with 25 ppm input sodium nitrite, they apparently feel justified only in recommending a reduction under manufacturing conditions to 75 ppm from the presently used levels in the US of 156 ppm, presumably to allow for the variability which must be expected in practice. Wierbicki, Heilgman and Wasserman's paper contains some interesting information on other matters besides the effects of irradiation, to be seen in the data relating to the control, non-irradiated, bacons and hams. The beneficial effect of ascorbate on the stability of the cured colour to fading in air was predictable from the work of a number of other investigatiors, as was its lack of effect on the intensity of colour in products where sufficient nitrite was available for this purpose (5,6,7).

At this point I would like to draw attention to an important difference between bacon as made in the United States and Canada and bacon as made in Great Britain, including Ireland, or in Denmark and Poland for export to Britain. In the latter countries the majority of bacon is made without a pasteurising process at the time of production whereas American and Canadian bacon is almost universally

heat-treated. A high proportion of British-type bacon is not even given the mild heat treatment which accompanies smoking. This means that in American bacon the cured meat pigment is fixed at an early stage in the denatured form, nitrosyl myochrome or myochromogen, whereas in British bacon it remains in the undenatured form, nitrosyl myoglobin, throughout storage and until the time of cooking for consumption. In addition, the majority of bacon sold in Britain is not vacuum packaged but is offered for sale in the sliced form exposed to the air. The action of added ascorbate on the colour of the bacon is quite different in these two different sets of conditions:

1. There is abundant evidence in the literature that the stability of the denatured cured meat pigment, in vacuum or in air, is enhanced by ascorbate. The stability of the undenatured pigment is also enhanced in the absence of air but in the presence of air the uncooked colour may be completely destroyed by as little as 100 - 200 ppm of ascorbate in one day or less. (5,6,7).

2. In British-type bacon undenatured pigment is converted into the denatured pigment at the time of cooking. Work in our laboratory indicates that the new colour is not formed unless a supply of nitrite oxide is present at that time. Thus any factor which accelerates the depletion of the residual nitrite level in the raw bacon, which ascorbate certainly does, accelerates the production of a bacon which may be red before cooking but will be grey after cooking.

For these reasons the major bacon-consuming countries should have some reservations about current recommendations to use moderate or large quantities of ascorbate in bacon in order to reduce nitrosamine formation and residual nitrite levels.

Wierbicki, Heiligman and Wassermann quote results from Fiddlers' group, now in the press, which indicate that nitrosamines, known to be formed preferentially in the fat in fried bacon, may arise through interaction of nitrite with the proteins of the fat connective tissue. Wierbicki, Heiligman and Wassermann show in their Table Vlll that in bacon fried twice - that is fried again after what they call 'pre-frying' the level of nitrosopyrrolidine was lower in the drippings after the second frying than after the first, but that in the bacon it was higher. No explanation is offered for this.

They also conclude that the presence of nitrate in the cure excercised a beneficial effect on the stability of the colour of ham (which would be the denatured pigment) and also on rancidity development. Their analytical figures do not indicate that these effects can be ascribed to the formation of additional nitrite from the added nitrate. They observed that meat may contain appreciable concentrations of nitrate before the addition of any curing salt and note that this must be allowed for in any regulations which purport to control the use of nitrate in curing. I must agree with this view: we have found the nitrate content of uncured meat to vary appreciably, from zero to 80 ppm in samples we have examined to date.

Let us now turn to paper F1 by Frouin, Thenot and Jondeau from the French Olida-Caby Company. These workers have continued

their studies, some of which were reported in 1973 in Paris,[8] on the properties and composition of the meat pigments. They used a particular pigment, made from meat hemoglobin, which they call nitrosoheme. They consider that nitroso myo- and hemoglobin, nitroso myo- and hemochromogen are four names for one single substance, nitrosoheme.

In Figure 2 I have set out the structure of some of the relevant heme pigments, simplified to show only the relationship of the iron atom to the ligands in co-ordination positions 5 and 6 and omitting the porphyrin ring and most other details. Frouin, Thenot and Jondeau's view appears to be correct if one considers only the substances which may be separated from cured meat using aqueous acetone, for instance by the Hornsey method. These are not the same substances as those present in the meat before extraction. Nitrosyl myoglobin contains undenatured protein attached to the iron atom by an imidazole group. In nitrosyl myochrome or myochromogen the protein is denatured and differences in behaviour associated with this difference in the protein have just been remarked upon. Myochromogens or hemochromogens may also be made in which this ligand consists not of protein but of a simpler molecule such as pyridine or another nitrogenous compound. All of the protein containing substances however go into acetone solution in the form of nitrosoheme, having lost their associated protein, and it is this nitrosoheme, in acetone solution, which was examined by Frouin's group. Its structure is uncertain; Tarladgis considered it to contain two nitrosyl residues.[9] Frouin and Cordier believed that one ligand might be an ascorbyl or cysteinyl residue when ascorbate or cysteine had been used in producing the colour.[8] It also appears that acetone is

bound to the structure in some manner.

In the analysis of cured meat pigments, fading of the acetone-water extract can be very troublesome, so this study of the mechanism of fading is of great analytical interest, but some caution is necessary in extrapolating from these results to the behaviour of pigments in meat. This said, the results of these experiments with nitrosoheme, confirmed to some degree in a specimen of cured meat, raise some most interesting questions. The authors allowed a solution of nitrosoheme to fade under the influence of light and observed the formation of a black precipitate. If this precipitate was left in the solution the fading of the pigment appeared to be temporarily delayed after about 6 hours whereas if it was removed by centrifuging at intervals the loss of pigment followed a logarithmic curve (their Fig. 2). It is suggested that in the absence of centrifuging, suspended material may increase the optical density of the solution before ultimately setting out as the black precipitate. The authors have carried out an elemental analysis and find that the molecule contains an iron atom and one nitrogen atom, which they consider to be probably in the form of a tightly bound nitrosyl group, not available for nitrosation reactions. The molecular weight is only about one half that of nitrosoheme and they conclude it may have been formed by the rupture of a pyrrole ring.

They obtained a reaction to the ferrocyanide test for ferric iron when fading was in progress, but not before, and were able to show a similar behaviour in a sample of ham during fading. They analysed the iron content of the precipitate colourimetrically using potassium thiocyanate (their Fig.4) and of the filtrate by an unspecified conductimetric method (their Fig.5), and in each case the curve of concentration against time shows a marked point of

inflexion at about 1¼ hours.

They postulate that on oxidation of the heme pigment, ferric iron is temporarily liberated and the pyrrole ring broken, then later the iron re-combines with a fragment of the broken pyrrole ring to form the black precipitate. They point out that ferric iron is known to catalyse oxidation both of the heme pigments and of fats, and the scheme they postulate may be a convenient explanation of the autocatalytic nature of these oxidations in cured meats.

There remain, however, some difficulties in reconciling the data given; for instance why does the postulated interference of the unprecipitated black substance with the fading curve (Fig. 2) occur only several hours after the iron content of the solution has passed its maximum and in the precipitate has reached its maximum (Figs 4 and 5).

Finally we have the remaining papers by Russian authors. Lyaskovskaya and Safronova (Paper F3) report in the changes in nucleotide and nucleoside content of a heated curing sausage during the manufacturing process, measured by ion-exchange chromatography. The nucleotide content fell during curing and markedly in the final heating stage. The nucleosides and bases remained roughly constant through the whole process, according to their Table 11, in which the concentrations are calculated on the dry basis. The changes are similar in kind to those reported by Rhodes (10) for irradiated meat but occur more quickly. Unfortunately no control experiments were done by Lyaskovskaya and Safronova with uncured meat so it is not possible to conclude whether this difference may be due to the curing process or to some other difference in the Russian conditions.

The contents of inosine and guanosine monophosphates, generally considered to be contributors to meat flavour, fell by a factor of about 4 during the production process. In his work, Rhodes observed that, unlike the case of fish, flavour changes in the meat could not be clearly associated with changes in the IMP Content, and the full significance of this Russian data is not clear. (Rapporteur's addendum:

During the meeting a colleague of the authors helpfully clarified this point. The results of their experiments have led to the following ideas for modifying the manufacturing process to produce a boiled sausage of improved quality.

- to use brine injection or perhaps dry curing instead of immersion curing, in order to minimise leaching and retain concentrations of flavouring compounds or their precursors.
- to replace boiling in water with other methods of heating, for the same reason).

The contribution from Shiskina, Yourtchenko and Veselovsky, Paper F5, is most interesting. Starting from the principle that free radicals emit quanta of light when they re-combine or disproportion, they set out to measure the chemiluminescence of oxidising fats, using appropriate photodetectors, amplifiers, integrators and recorders. They state that the chemiluminescence due to radicals formed on heating is a measure of peroxide present in the fat and that the quenching of the luminescence which occurs on electrolysis is a measure of the antioxidant activity of the fat. Two sets of instruments, identified in the paper by Russian codes, were set up to measure these too effects. They give the following details: The photomultiplier

in the electro chemiluminescence equipment operated at 1400 volts. Electrolysis was carried out in solution in sodium citrate + methanol in a cell with stabilised voltage. Thermochemiliuminescence was measured in a cell at approximately 80^{o}C. The lipids were extracted from various meat products with chloroform: methanol 2:1. Other details of the analytical technique are not unfortunately, given. Their Figure 1 shows the changes in thermochemiluminescence (Curve 1, rising from left to right) and quenching of the electrochemiluminescence (Curve 2, falling from the right) and the relationship of both to the storage time of the sausage with 10% fat content from which the fat samples were extracted. Their Figure 2 shows the correlation between peroxide value and thermochemiluminescence (in arbitary units). These curves indicate reasonable agreement between the luminescence technique and both the conventional peroxide value and the expected behaviour of a stored fat. Their Figure 3, in confirmation, shows a good correlation between thermochemiluminescence and organoleptic score for the same meat product. The authors claim that this method may be used to measure peroxide values down to 10^{-7}M. If this claim can be substantiated on more intensive tests this may prove to be a most useful tool for the examination of fats, especially during the induction period when present methods of peroxide analysis yield little useful information about the probable storage life of a fat.

References

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FIG 1

Fate of 150 ppm $NaNO_2$ in pork, after heating at 71°C
(After Sebranek et al 1973)

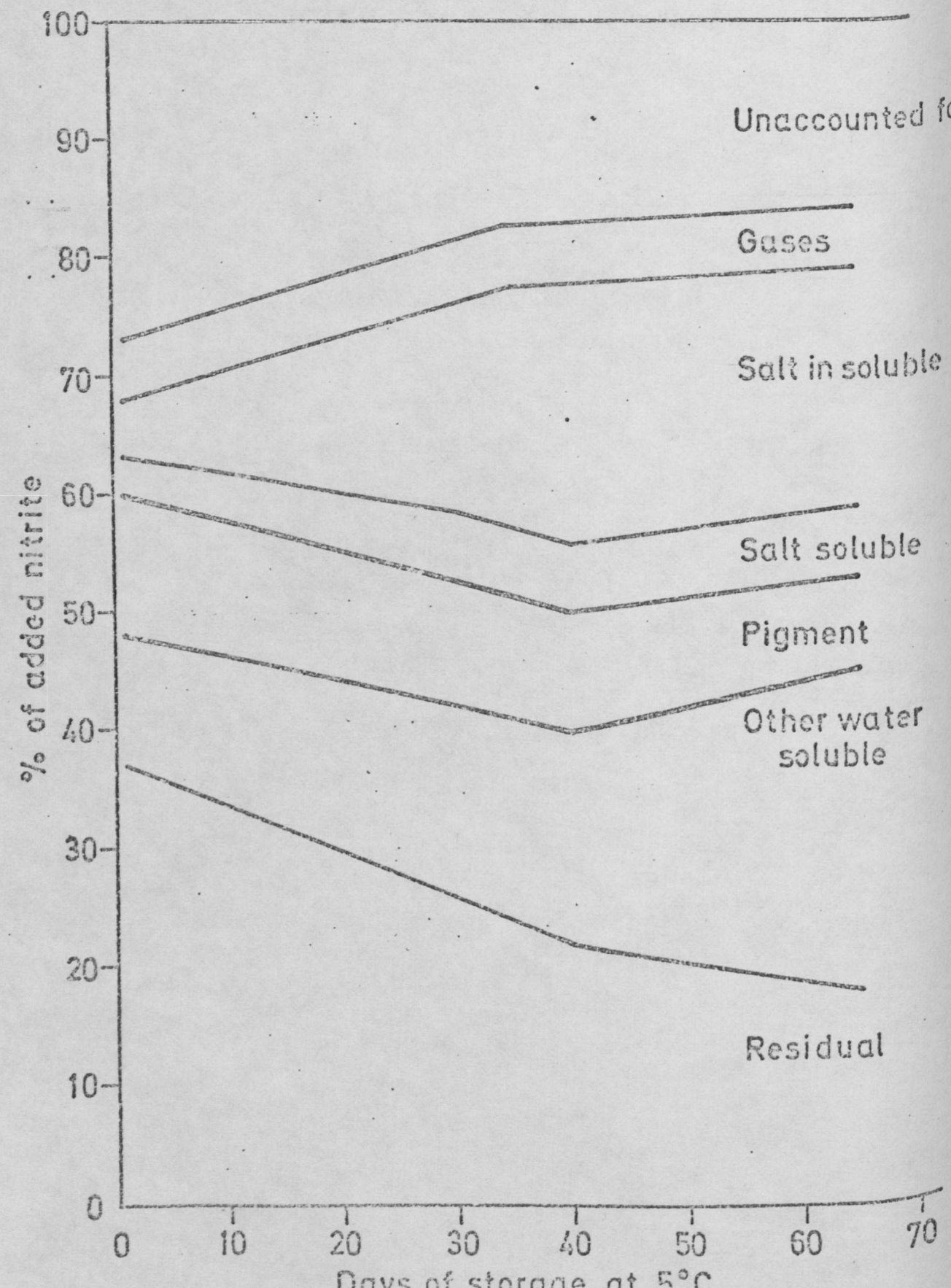

FIG 2

MEAT PIGMENTS

Myoglobin

native protein
|
>Fe<
|
H_2O
|
native protein

Nitrosyl myoglobin

native protein
|
>Fe<
|
NO

Nitrosyl myochrome (hemochrome) (-chromogen)

denatured protein
|
>Fe<
|
NO

other N cpds
|
>Fe<
|
NO

Nitrosoheme

reductant
|
>Fe<
|
NO

(Frouin & Cordier 1973)

NO
|
>Fe<
|
NO

(Tarladgis 1962)

?
|
>Fe<
|
NO

Hemin chloride

HCl
|
>Fe<
|
HCl

Hematin

H_2O
|
>Fe<
|
H_2O