REACTIVITY OF HAEMATIN IN PROCESSED MEATS

D.A. LEDWARD, WENDY J. SCHOFIELD, T. HAZEL and R.J. NEALE

University of Nottingham, Nottingham, England.

INTRODUCTION

THE REACTIONS undergone by the haemoproteins of meat (mainly myoglobin) during storage, heating and curing have been extensively researched and comprehensively documented. However there is increasing interest in the development and utilisation of freeze-dried and intermediate(I.M.) meats and although it is well established that the freshly prepared products have the pigments in the same form as their full moisture counterparts (Lawrie, 1979, Obanu and Ledward, 1975) there is some doubt as to their behaviour during storage. Some workers claim that the haemoproteins of freeze-dried meats degrade to bile pigments (Lawrie, 1979) while Obanu and Ledward (1975) suggest that in I.M. meats the haematin remains intact, but firmly bound to the meat matrix.

In view of the importance of the haemoproteins and their environment, to both the colour and nutritional quality of meat (Hazell et al, 1978) a study of their stability in dried and I.M. beef was undertaken.

MATERIALS AND METHODS

A BEEF l.dorsi muscle, freed of visible fat and connective tissue, was cut in cubes ($\sim$1cm^3) and samples either
(a) freeze dried
or (b) cooked in boiling water for 1 hour and freeze dried
or (c) canned by hot filling and processing in water at 260°F for 45 minutes; the meat to water ratio being 70:30
or (d) mixed with 1.5 times their weight of infusing solution (515g water, 394g glycerol, 95g salt and 5g potassium sorbate), heated at 100°C for 15 minutes in cans, which were then sealed and heated at 100°C for a further 15 minutes. After equilibration this yielded meat of a_w 0.85 (Obanu et al, 1975). Half the cans
(e) were opened and the cubes dabbed with absorbent tissues to remove surplus fluid.

Samples (a), (b) and (e) were packed in contact with air in PVDC Cryovac and together with samples (c) and (d) were stored at -12 and +38°C.

Spectral Analysis

The reflectance spectra of intact meat pieces was recorded against MgO using a Perkin-Elmer Model 124 spectrophotometer fitted with a reflectance attachment. The range scanned was 700 to 370nm. The same instrument, set up in the transmission mode, was used to record the absorption spectra of solutions at a path length of 1cm.

Solubility of Iron and Porphyrin

Although water and dilute salt solutions will readily extract the haemoproteins of raw meat in cooked meats the precipitated cooked meat haemoproteins can only be solubilised with difficulty (Ledward, 1971). Preliminary experiments using 40% pyridine, 6M area, 3% sodium dodecyl sulphate plus 1% β-mercaptoethanol, 0.1N HCl and N and 5N NaOH indicated that 0.1N HCl and N.NaOH were the most effective in extracting haematin from cooked meat.

Preliminary work also indicated that the extraction itself could lead to destruction of haematin from canned and I.M. meats. The most successful technique was to freeze dry the meat just prior to analysis followed by gently powdering in a pestle and mortar and then treatment with the appropriate solvent at 2°C for 48 hours. In all cases the ratio of protein:solvent was about 1:10.

Iron concentrations were determined by atomic absorption spectrophotometry (EEL Model 240) and porphyrin concentrations by reaction with benzidine (Hazel, 1979).

Porphyrin concentrations of the solution were calculated as a percentage of that extracted from the raw meat by N.NaOH and iron concentration as a percentage of the iron content of the ashed freeze dried starting material.

RESULTS

TOTAL iron content of the beef used was 7.89 mg/100g dry matter with a standard deviation of 0.08 on 12 samples and did not change significantly during processing and storage. Due to the presence of glycerol and salt, the iron content of the I.M. meat was 3.90 $\pm$ 0.13 mg/100g dry matter which remained constant during storage.

Effect of Storage at 38°C on Reflectance Spectra

In Figure 1 the reflectance spectra of freshly prepared freeze dried raw beef is shown together with the spectra

of the samples stored in air at 38°C for both 4 weeks and 20 weeks. It is seen that the characteristic spectra of the fresh meat (predominantly oxymyoglobin) is lost during storage. During storage of cooked freeze dried meat under the same conditions the characteristic cooked meat haemoprotein spectra was lost. In agreement with the earlier work of Obanu and Ledward (1975) the I.M. meat also rapidly lost its haemoprotein characteristics during aerobic storage at 38°C (Fig. 2). However the anaerobically stored meat samples (both canned and I.M.) showed no loss of haemoprotein character during 20 weeks storage at 38°C (Fig.2). In I.M. meat the loss of haemoprotein character appears to be rapider in heat treated samples (May, 1977).

Solubility in Acid and Alkali of Porphyrin and Iron in the Meats.

In all cases the majority of the iron (> 80 %) in the meats was extracted, in both the freshly prepared and stored meats, on treatment with either 0.1N HCl or N.NaOH. However during air storage the amount of porphyrin solubilised decreased.

The results for the alkali extraction of cooked and raw freeze dried beef are shown in Fig. 3 where it is seen that at -12°C the raw pigment appears to be more stable than the cooked.

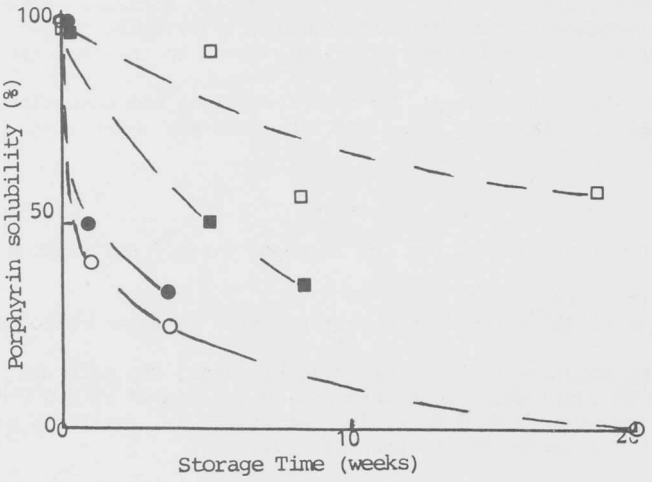

FIGURE 3. Porphyrin solubility (as a % of that extracted from raw beef) in N NaOH from raw freeze dried (○,□) and cooked freeze dried (●,■) during storage at 38°C (○,●) and -12°C. (□,■).

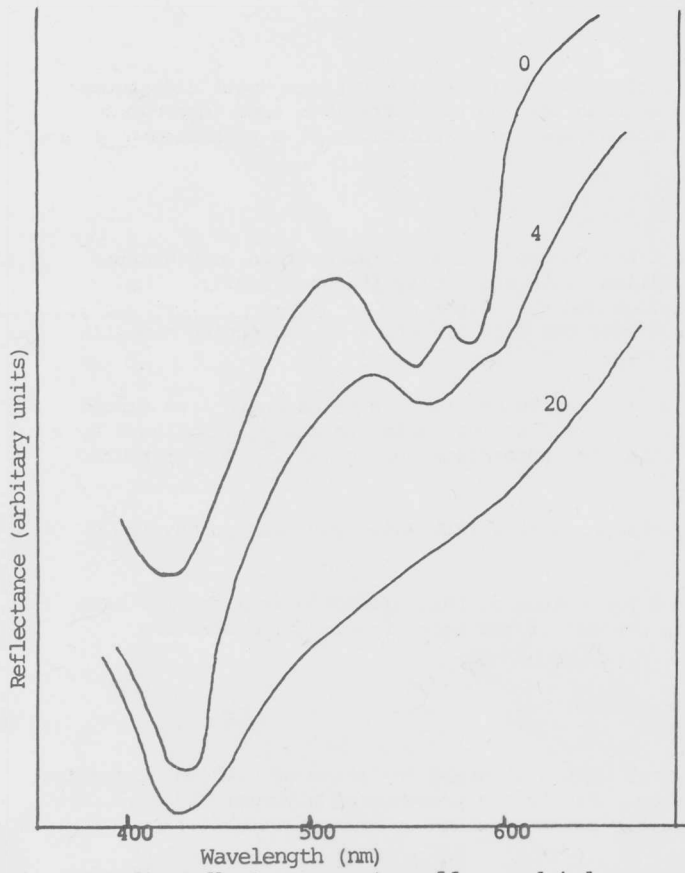

FIGURE 1. Reflectance spectra of freeze-dried raw beef during storage at 38°C. The superscripts refer to the number of weeks of storage.

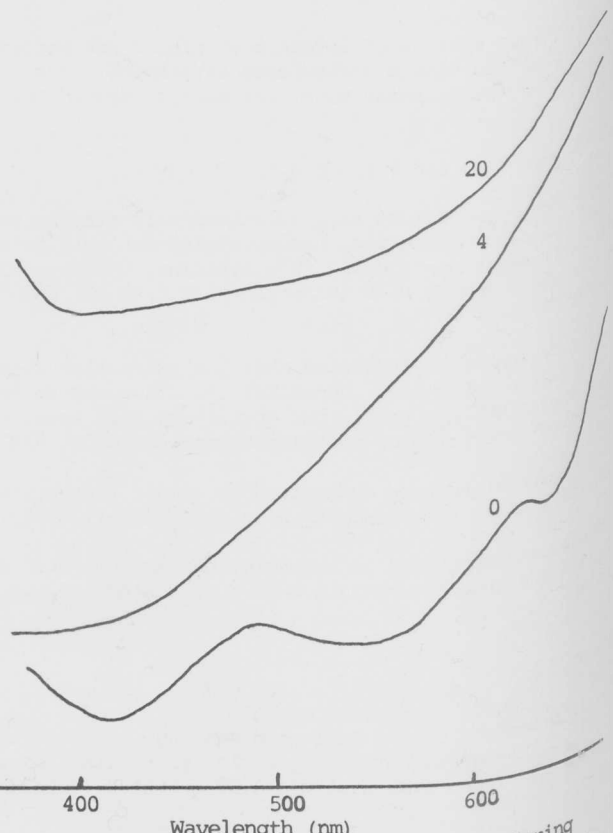

FIGURE 2. Reflectance spectra of I.M. beef during storage at 38°C. The superscripts refer to the number of weeks of storage. The spectra did not change during anaerobic storage.

With the acid extraction the porphyrin is less stable as, although all of it can be extracted in the freshly prepared freeze dried meats (both raw and cooked) only about 5% (mean of 4 samples) was extracted from the cooked sample after 5 weeks at -12°C. From the raw sample 30% (mean of two samples) was extracted by acid after 5 weeks at -12°C and none (mean of two samples) after 8 weeks at this temperature. After 4 weeks at 38°C no porphyrin, from either the raw or cooked freeze dried meat, could be extracted by acid (4 samples analysed).

Spectra of the solutions obtained verified these observations as marked haematin character (Lemberg and Legge, 1949) was seen in the acid and alkaline solutions from the freshly processed meats but significantly less, or none at all, in the stored freeze dried meats.

Even with the mild extraction procedure used in the present work it proved impossible to solubilise all the porphyrin from even freshly prepared I.M. meat. Less than 30% was extracted with N NaOH (values of 17,0, 30 and 30 being found for 4 samples) even though most of the iron was soluble (mean = 88 % on 4 samples). In 0.1N HCl extracts it was not possible to detect any porphyrin in 4 samples although the iron solubility was high (> 90 %).

These degrees of extraction were apparently independent of storage time or temperature for the anaerobically stored samples but for the I.M. samples stored in air no porphyrin was extracted after 20 weeks at -12°C or 4 weeks at 38°C. Unfortunately no analyses were made at shorter time intervals.

In canned meat, removed to air storage after processing and stored at -12°C for 20 weeks about 50% (mean of 2 determinations) of the porphyrin could be extracted in NaOH compared to over 80% at zero time.

In all solutions from the I.M. and canned meats the presence or absence of haematin compounds was supported by the absorption spectra; solutions giving positive porphyrin tests always displaying maxima in the Soret region.

DISCUSSION

THE RESULTS reported above strongly suggest that during air storage the haematin pigments of freeze dried, I.M. and cooked meat are degraded to non-haematin compounds. It is possible to explain the changes observed in the reflectance spectra as being due to the formation of 'free' haematin (Obanu and Ledward, 1975). However the absence of porphyrin in the alkali and acid extracts from the stored meats argue strongly against this as haematin complexes should be stable in these solvents (Lemberg and Legge, 1949).

As the oxidation takes place in well heated meat and at sub-zero temperatures it is unlikely to be caused by either enzymes or bacteria. This non-enzyme oxidation of the haematin is apparently more rapid in cooked compared to raw meats, at I.M. water activities and at elevated temperatures.

The I.M. beef used in the present study was infused with glycerol which often contains certain reducible impurities and these impurities have been shown to totally destroy the haematin of myoglobin even when the protein was not denatured (Bello and Bello, 1976). However freeze dried meat does not contain glycerol but it does have a large surface area exposed to air and this, together with its low a_w (≈0.6) will increase its susceptibility to lipid oxidation and it has been shown that the free radicals and hydroperoxides formed during the oxidation of muscle lipids can destroy the haematin ring in haemoproteins (Roubal and Tappel, 1966). Support for the hypothysis that the intermediates formed during the oxidation of muscles lipids are responsible for the haematin destruction in these processed meats is given by the following observations; (i) lipid oxidation is maximal in the I.M. moisture range i.e. at a_w's at which haematin destruction is most rapid although of course glycerol impurities may also contribute in these samples (ii) freeze dried whole blood shows little loss of haematin character during air storage (Hazell, 1979) and (iii) the rate of destruction is faster in cooked compared to raw meat (either freeze dried or full moisture) and lipid oxidation is known to be more rapid in cooked than fresh meat.

Fortunately this loss of haematin does not appear to affect the high nutritional availability of the iron associated with meat (Obanu et al, 1976, Hazel, 1979).

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