PHYSICAL AND CHEMICAL CHANGES IN PROTEINS DURING THE MATURATION OF PARMA HAM.
II. COLOUR

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It is widely accepted that a correct production technique does not need to employ nitroso-forming substances to produce a uniform and deep red colour in Parma ham. In effect a great proportion of this pork product has been produced since remote times without nitrate but with sodium chloride only.

In spite of such lack of colour-forming additives, the colour of properly matured hams cannot be described in the same way as for fresh meat. Ham colour changes from light pink at the beginning to deep red at the end of maturing and has an unexpected stability even to heat treatments which would brown fresh meat colour.

The most plausible hypothesis is that the ham colour is due to a low-spin octahedral complex in which iron is linked to an electron donor, π-acceptor group, with characteristics similar to those of NO-myoglobin. According to this model, iron could be in one of the following positions:

1) - in the centre of native myoglobin, held as Fe^{2+} by the unimpaired reducing systems of the muscle. Such myoglobin would become oxygenated in the air, producing MbO_2. The deep red colour of the ham would be due only to the higher concentration of myoglobin consequent to muscle dehydration:

2) - as Fe^{2+} or Fe^{3+}, in the centre of an octahedral complex in which iron would coordinate an aminoacidic residue of globin.

Ascertaining the valence of iron in the pigment would make easy the distinction between such hypotheses. This can be accomplished by spectroscopic (electronic and Mossbauer spectroscopy) and magnetic studies of myoglobin but, though indirectly, also from the determination of muscle reducing capabilities.

The existence of reducing systems in muscles has been first suggested by Dean and Ball (1960) who observed that metmyoglobin present on meat surface can be reduced by exposure to air.

This capability, termed ARA (aerobic metmyoglobin reduction activity), has been confirmed by other studies (Giddings,1974). Similarly, it has been shown that muscles can reduce metmyoglobin in anaerobic conditions as well, (metmyoglobin anaerobic reduction activity, or MRA) after oxidation and activation with ferricyanide. Both ARA and MRA have been recently discussed and reviewed (Giddings, 1977; O'Keeffe and Hood, 1982).

It has been reported that fresh beef MRA is inhibited by salt concentrations similar to those present in matured hams (Stewart et al.1965). This fact, if confirmed in the case of Parma ham, could give useful information in the study of the chemical state of muscle pigments based on their spectral properties. This paper reports the first results of ham colour studies based on the analysis of visible spectra and of reducing activities and is a part of a wider investigation on the protein changes in the maturation of Parma ham (Bellatti et al., 1983).

EXPERIMENTAL PART

Analyses were carried out on biceps femoris muscles, obtained from swine thighs salted and matured as described elsewhere (Bellatti et al. 1983). The times of the analyses, together with the corresponding abbreviations, are here reported:

F = fresh (i.e.prior to salting); S = post-salting; R = post-resting; MS = half-maturing (i.e. end of the 6th month); ST = end of maturing (i.e. end of the 12th month).

Pigment analysis

Metmyoglobin (MMb) and total myoglobin (Mb) content of muscles were determined spectrophotometrically according to the method of WOLFE (1978). This method enables to determine separately MMb (recording the absorbance at 630 nm of the aqueous extract) and total ferrous derivatives of Mb (absorbance at 578 nm of the same extract after bubbling carbon monoxide for 10 min.). The extracts proved stable for 2-3 hours, according to the Authors.

Measurement of Metmyoglobin reducing activities

ARA

The mid part of biceps femoris muscle was sliced into four steaks 1 cm thick and two steaks were left for 48 h at 0°C in a low oxygen atmosphere (CO_2/O_2 = 99/1); after measuring the colour in the way described below, the slices were packaged in an air-permeable bag and stored at 0°C for 48h, then the colour was recorded again.

The remaining two slices were measured after storage in air at 0°C for 96h and their colour referred to as the best obtainable by the meat under investigation.

The measurement of colour was performed by the Gardner meter, recording the Hunter "a" value of the steaks wrapped in a transparent film and placed on the glass plate of the colorimeter, which had been standardized against a reference plate with the following specifications:

L = 67.8 ; a = 17.6 ; b = 9.2

ARA was expressed by the ratio:

$$\frac{a_{\text{after 48h in } O_2} - a_{\text{after 48 h in } CO_2}}{a_{\text{after 96 h in } O_2} - a_{\text{after 48h in } CO_2}} \times 100$$

MRA

Anaerobic reducing activity was measured as the capability to restore the red colour by the tissue oxidized with ferricyanide and stored anaerobically at 37°C to activate reducing systems.

The ratio a/b of Hunter scale was recorded for each sample by the Gardner meter standardized as described for ARA. Measuring MRA by this ratio resulted in improved sensitivity, since b decreases and a increases as long as the red component is anaerobically restored (unlike ARA, where b behaves like a constant).

Samples were prepared in Cryovac bags, mixing 50 g of the muscle, previously minced through 2 mm diam.holes, with 3 ml of 0,1% in 0,01 M HCl Chlortetracycline and with 5 ml of 0,2% $K_3Fe(CN)_6$; after vigorous shaking, each bag was vacuum-sealed and dipped into a thermostatic bath at 30°C in the darkness; 30 min.later the mixture was poured in a

uniform layer into the Gardner tray fitted for the analysis of liquid samples. The ratios a/b were recorded at 5 minutes intervals, leaving the tray at ambient temp. after each measurement. MRA was expressed as the reciprocal of the time required for a 0.100 units increase of the ratio starting from its minimal value. This arbitrary criterium was chosen after observing that such an increase roughly corresponds to a 50% formation of reduced myoglobin (correlation with spectrophotometric analysis was significant at the 99% level).

RESULTS AND DISCUSSION

In can be seen from Table 1 that both ARA and MRA disappear during the first phases of processing confirming in this way the inhibiting action of sodium chloride (Stewart et al.,1965).

Such a loss of muscle reducing activity agrees with the decrease of SH groups observed by Bellatti et al. (1983) and with previous reports of a close relation between SH groups and activation of metmyoglobin reductase in dolphin muscles (Matsui et al., 1975). Myoblobin is concurrently oxidated as is clearly shown in Table 2 where metmyoglobin is expressed as percent of total muscle pigments. It is important to notice, though, that in group 1 metmyoglobin reaches a maximum with phase 3 (post-resting) when NaCl content begins to be fairly high (~ 2%) while in group 2 metmyoglobin increase is delayed according to the milder curing technology.

It would appear from Table 2 that myoglobin suffers more from a high salt level than from a longer refrigeration period. But what is more interesting is the apparent tendency of metmyoglobin to be reduced during the last phases of maturing in spite of the loss of muscle reducing capacity (Table 1).

A possible key for the understanding of this phenomenom can be traced in the visible spectra of water extracts used to measure metmyoglobin concentration (Fig.1). Such spectra show two main features related to processing:

1) a gradual decrease, from post-resting onwards, of the metmyoglobin band at 630 nm typical of the H_2O-Fe^{3+}bond;
2) a deformation of the α-β system of MbO_2 with an increase of the 540 nm band and a corresponding decrease of the 580 nm one. Moreover, the latter band is splitted, starting from post-resting, being coupled with a shoulder at 595 nm which grows steadily up to the end of maturation to the point of surmounting the 580 nm band.
It has been reported (Antonini and Brunori, 1971) that low-spin ferric-myoglobin complexes with deprotonised ligands lack the 630 nm band and show two bands between 500 and 600 nm instead of a single one at 500 nm as does metmyoglobin. This is the cause for the red colour of low-spin ferric complexes. The visible spectra of the matured hams could therefore express the formation of a coordination compound of Fe^{3+} which is already present at post-resting, with a basic amino acid residue of globin. Such a residue cannot be imidazole since the spectrum points out a shift to red side of the α and β bands while coordination with imidazole would have caused a shift towards the blue.

The preceding comments, leaving aside the problem of Fe^{3+} ligand, imply that the decrease of the 630 nm band has lead to an incorrect assessment of metmyoglobin concentrations.
This means that, from the moment in which the spectrum begins to deform, ferrimyoglobin cannot be estimated as a coordination compound with water since water might have been removed by dehydration and substituted with a different ligand. Therefore metmyoglobin low values at end-maturing cannot be taken as real.
To conclude, although this study has not produced evidence on the chemical nature of the compound responsible for the red colour of the matured hams it has given useful indication on future research lines. The investigation is continuing with Mossbauer spectroscopy and magnetic properties evaluation of ham pigment.

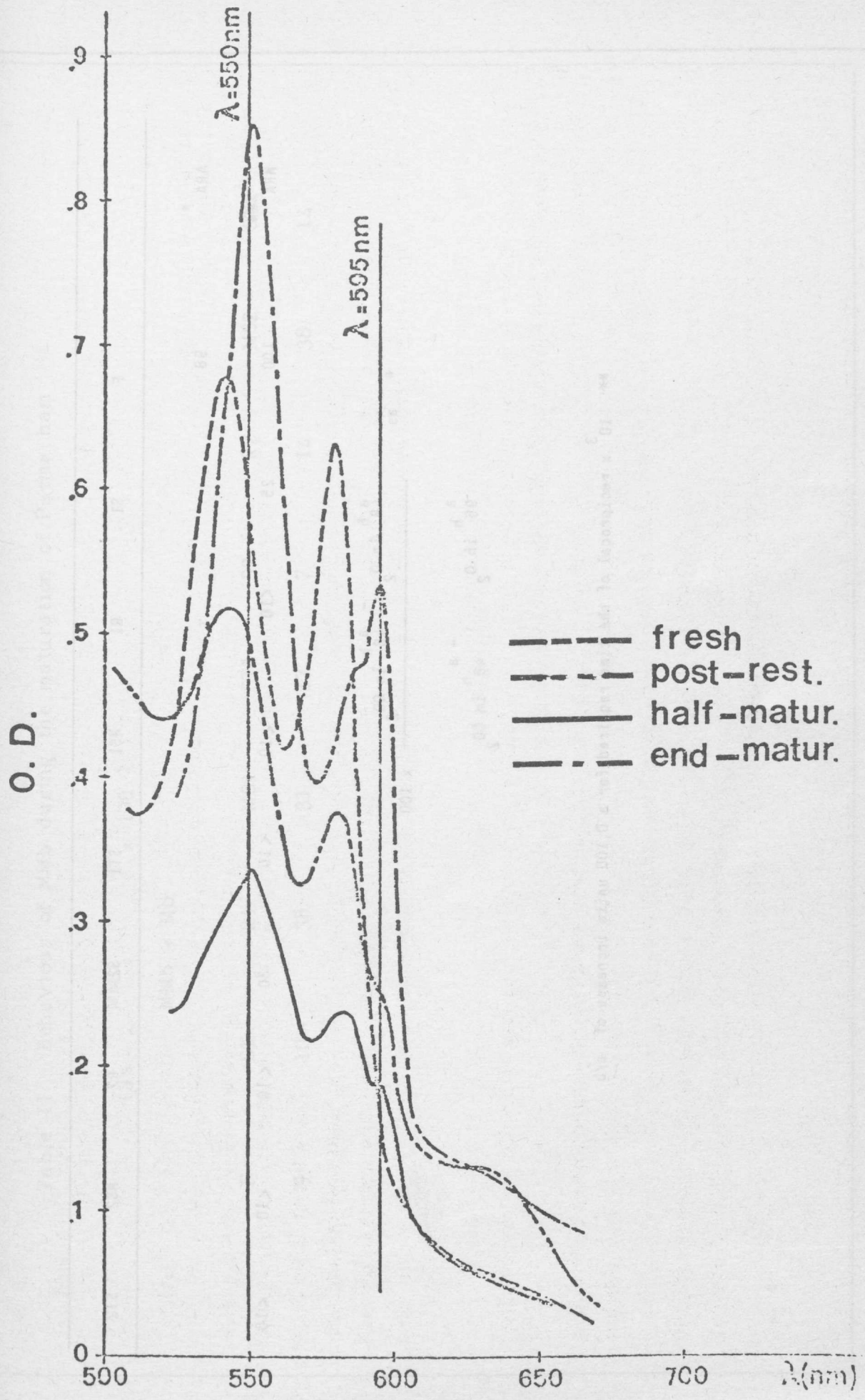

Fig. 1 - Absorbance spectra of hams extracts at several steps of maturing.

Table 1

	F	S1	R1	MS1	ST1	S2	R2	MS2	ST2
ARA *	98	–	–	–	–	–	–	–	–
MRA **	100	25	<10	<10	<10	30	<10	<10	<10

* as $\dfrac{a^{h}_{48}\ \text{in}\ O_2 - a^{h}_{48}\ \text{in}\ CO_2}{a^{h}_{96}\ \text{in}\ O_2 - a^{h}_{48}\ \text{in}\ CO_2} \times 100$

** 10^3 x reciprocal of the time required for a 0,100 units increase of a/b

Table II Behaviour of MMb during the maturation of Parma ham

$$\left(\text{as } \frac{\text{MMb}}{\text{MMb + Mb}} \times 100\right)$$

F	S1	R1	MS1	ST1	S2	R2	MS2	ST2
9	10	38	31	17	7	14	38	14

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MODIFICAZIONI FISICHE E CHIMICHE DELLE PROTEINE NELLA MATURAZIONE DEL PROSCIUTTO DI PARMA

II - Colore

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Il prosciutto di Parma sviluppa, nel corso della maturazione, un color rosso intenso, stabile al taglio, anche in assenza di nitrato aggiunto. La formazione del colore può essere dovuta alla stabilizzazione della mioglobina ad opera di sistemi riducenti specifici o alla coordinazione del ferro da parte di un legante esterno, con formazione di un complesso ottaedrico a basso spin, stabile.

Queste ipotesi sono state esaminate studiando il comportamento spettrale dei pigmenti muscolari di 36 prosciutti, preparati senza nitrato nè nitrito e analizzati in diverse fasi della maturazione. I risultati indicano una modificazione chimica del pigmento, ma non permettono di definirne l'esatta natura chimica.

Physical and Chemical Changes Occurring in Proteins during the Maturation of the Parma Ham

II - Colour

The Parma ham develops on maturing, even in the absence of added nitrate, a deep colour which is stable also after slicing. The colour may be due to specific myoglobin-reducing systems or to iron-binding external ligands capable of forming a stable octahedral low-spin complex.

Both hypotheses were investigated in this study, where the spectral behaviour was examined of the muscle proteins taken, at different stages of maturation, from 36 hams prepared without nitrate or nitrite. The results indicate a chemical change in the pigment, but do not allow the precise chemical nature of the modified pigment to be defined.