SPECTROPHOTOMETRICAL BEHAVIOUR OF NITRATE FREE AND NITRATE CURED MUSCLE EXTRACTS.

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Keywords: ham, nitrate free, low nitrate adjuncts, myoglobin complexes, UV spectrophotometry

Background and objectives:
Nitrate curing is generally used to achieve stable red colour in dry-cured meats such as dried hams. However, red colour in Parma hams develops naturally from meat myoglobin during maturation as unique consequence of salt addition and dehydration.
Molecules containing electron donor atoms (mainly nitrogen) are generated during maturation, particularly peptides and amino acids resulting from extensive protein breakdown (1), and they might play a role as heme ligands in myoglobin stabilisation (2). Spectroscopic studies made so far with the aid of EPR (3) and absorption spectrophotometry (4) have failed to demonstrate the chemical structure of the pigment responsible of red colour in nitrate free hams, but there is evidence that it differs in nature from nitrosylmyoglobin. This pigment is likely affected by the modified chemical environment resulting from combined dehydration and protein breakdown. Notably, it might be modified to interact with lipophilic compounds (amino acids, peptides) yielded by process-related chemical changes.
Therefore, we studied the spectral behaviour of nitrate-added, nitrate free and low-nitrate-added dry-cured hams as extracted with solvents having increasing lipophilic properties.

Methods:
Three batches of nitrate free (batch 1), nitrate-added (batch 2), 200 mg/Kg nitrate addition, and low nitrate-added (batch 3), 50 mg/Kg nitrate addition (N=25 per batch) 12-24 months old hams were collected and their central section, at the knee level (*B. femoris* muscle) underwent spectrophotometric analysis. Ham extracts were obtained through this series of increasing lipophilic solvents: 1-aqueous (phosphate buffer 0.2M pH=6); 2- acetone:water (75/25 v/v); 3- THF. Extraction procedure: muscles were homogenised with extractant 1in a 1:10 (w/w) muscle:solvent ratio, the slurry centrifuged and the solution filtered through Millex Durapore PVDF 0.45 µm filters. The solid was treated with extractant 2 in a 1:2.5 (w/w) solid:solvent ratio and the residue extracted with THF for 30 min in the same way.
The solutions from each extraction procedure were submitted to spectrophotometric analysis and absorbance spectra were recorded from 400-700 nm with a double array spectrophotometer (Jasco V550, scanning speed 200 nm/min, data pitch 0.2 nm), with the extractants as the blanks.

Results and discussion:
Water-extracted nitrate-free hams show maxima at 427, 551, 585 and 595 nm (fig.1), while acetone and THF yield spectra with absorption at 417, 544 and 584 nm. Spectra of nitrate added hams resemble their nitrate free counterparts when extracted with water and THF, while acetone extraction of hams results in characteristic nitrosylMb maxima (fig.2). It is noteworthy that the former ham class is least soluble in acetone, whereas the same extractant is most effective with the latter ham type. Therefore, the red pigment exhibits major differences in terms of solvent extractability, depending on whether the meat was treated with nitrate or salt only. Hams with low nitrate addition show the spectral pattern of both ham types (fig. 3), with reduced NOMb acetone maxima, suggesting that redness of dry-cured hams muscle is mainly due to NOMb when adequate amounts of nitrate are available, while in absence of nitrate a red pigment forms, having both hydrophilic and lipophilic behaviour and spectral properties in THF similar to those of MbO_2.

Conclusions:
Water soluble myoglobin complex in dried, nitrate-free hams is still unknown and its spectral behaviour is very different from that of water soluble muscle myoglobins, while spectra in acetone and THF resemble MbO_2 in its α–β doublet.
This study confirms that a rather hydrophobic heme environment develops on ageing, with Mb becoming more soluble in organic solvents.
This analytical procedure can be used to establish whether a dried meat sample has been added with nitrate, even if at very low concentration.

Pertinent literature:
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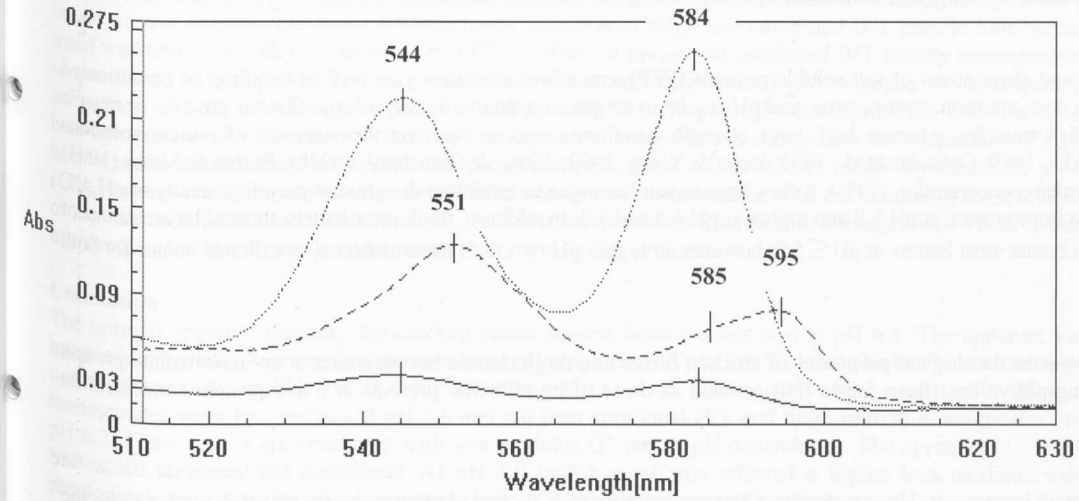
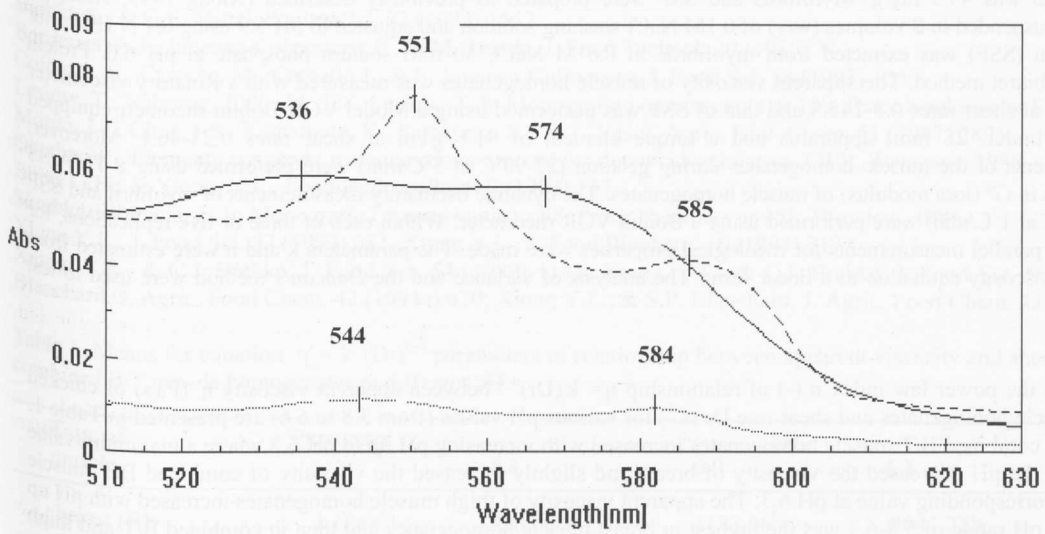
4) Morita H., Niu J., Sakata R. & Nakata Y. 1996. Red pigment of Parma ham and bacterial influence on its formation. *J. Food Sci*. **61**, 1021-1023.

Figures 1, 2, 3.

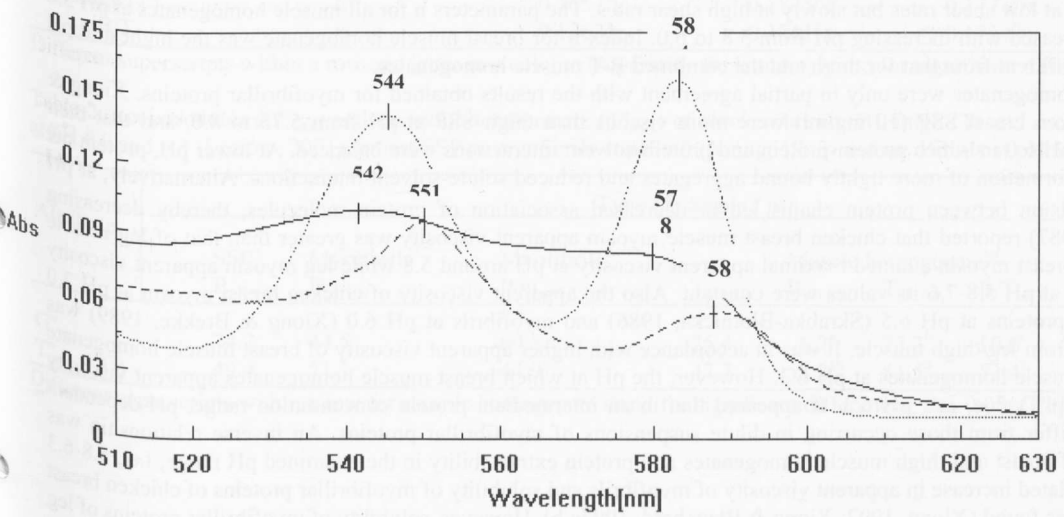
Visible absorption spectra of ham extracts in aqueous buffer (——), acetone (——) and THF (·····).



1. NITRATE FREE



2. WITH NITRATE



3. LOW NITRATE