# MYOGLOBIN LAYERS IN MEAT: A MULTISPECTRAL IMAGING APPROACH

**B. Hernández*, C. Sáenz, C. Alberdi, S. Alfonso, and J.M. Diñeiro**

*Departamento de Física. Universidad Pública de Navarra. Campus Arrosadía. 31006 Pamplona, Spain. Email: bego@unavarra.es*

**Keywords:** multispectral imaging, myoglobin, beef

## Introduction

Meat colour depends on several productive factors like breed, sex, animal age or pH (Seideman *et al.*, 1984) but once they are fixed, the main factor responsible of colour and colour changes is the evolution of the surface concentrations of haeminic pigments, particularly of myoglobin. Prior to being exposed to air, myoglobin is found in the reduced form (Mb). When meat is exposed to air, oxygen diffusion through the surface converts Mb to the oxygenated form or oxymyoglobin ($MbO_2$) and, with time, metmyoglobin (MMb) will appear after the oxidation of $MbO_2$. If enough time of exposure to air is allowed, a layered structure appears where MMb is found immediately beneath the surface, followed by a deeper layer of $MbO_2$ and an almost oxygen free region dominated by Mb. However, even for long times of exposure to air, layer thickness is of the order of a few millimetres (Renerre, 2004) and therefore beyond the capabilities of standard spectrophotometric instrumentation.

Presently a single method is available to determine position and width of myoglobin layers. The so called 'squash plate method' consists in squashing a meat sample between two transparent glass plates. Glass prevents blooming of the observed surface and oxygenation takes place along the direction parallel to the plates. In this way the development of pigment layers can be observed through the glass and their position and thickness measured (O'Keeffe and Hood, 1982; Powell and Macdougall, 1993; Gutzke *et al.*, 1997). Boundaries between pigments layers are determined by visual inspection, using a magnifier lens and their positions and widths measured with a precision ruler or a micrometer.

However, the overall process depends on the visual capabilities of the observer and several factors will limit the accuracy and usefulness of the results. Pigment layers are identified by their colour since each of the three pigment forms has a distinct colour. However boundaries between pigment layers are not sharp and colour transition between them is gradual, making difficult the determination of the end of one layer and the beginning of the next one. Pigment layers need to be completely developed to be discernible and the method does not work if a mixture of pigments coexists in the same region. In addition, colour differences between pigments may be subtle and measurements biased by the colour discrimination capabilities of the observer. Furthermore colour differences will depend on the illumination used during the measurement. This data is not provided in existing works but meat colour is known to be greatly affected by illumination.

In this work we use standard spectrophotometric methods combined with a multispectral imaging acquisition system to determine the layered structure of myoglobin derivatives during meat oxygenation.

## Materials and Methods

The multispectral imaging acquisition system consists of an objective lens, a CRI VariSpec™ Liquid Crystal Tunable Filter (LCTF), a relay lens and a 1392 x 1040 pixels, 12 bit, monochrome digital camera Evolution VF. The LTCF clear aperture is 20 mm and works in the wavelength range 400-720nm with 10nm of bandwidth. All the system is mounted in a Hama Repro XZ adjustable column with 4x100 W tungsten lamps for illumination. System optics was selected to work in macro mode to increase the spatial resolution. Reflectance calibration using a lambertian white diffuser allowed raw images to be converted in reflectance images and spatial calibration was performed to convert pixel values to distances.

Two 20 x 20cm square highly transparent glass plates formed the 'squash plate'. Plates were kept fixed and parallel using four 2cm long spacers. Both plates were screwed to the spacers making a solid and stable 'sample holder' where samples were squeezed and imaged through.

Meat samples were obtained from beef *longissimus dorsi* muscle. Samples of 2.2cm thick were cut from the inner part of the muscle to ensure that initially only Mb was present. Samples were immediately introduced between the squash plates. The border of the squash plate was covered with oxygen permeable film to allow oxygenation and prevent excessive dry of the sample.

Relative concentrations of myoglobin forms were obtained using the standard procedure (A.M.S.A., 1991) from reflectance images using (K/S) ratios at specific isobestic points (474, 525, 572 and 610 nm). In this way, images corresponding to pigment concentrations can be constructed.

A final false colour image is constructed assigning each concentration image to each of the RGB colour channels. In this way pigments can be easily identified.

## Results and Discussion

As an example of the resulting pigment concentration images, pigment layers after 1 hour and 8 hours of oxygenation time at room temperature (20°C) are shown in Figure 1. These images are B/W versions of the original colour coded images. In these images Mb appears darker (Mb covers most of the imaged area in this sample) and $MbO_2$ appears as a whitish band, broad and close to the image surface in the left image and as a somewhat narrower band, shifted towards the inner part of the sample, after 8 hours. MMb appears dark (slightly lighter than Mb) and immediately beneath the surface after 8 hours. MMb can not be distinguished in the B/W image corresponding to 1 hour of oxygenation, because concentration values of MMb are small, but not zero, at this time.

Both images have 1040 (width) x 1392 (height) pixels. The imaged area after spatial calibration is 1.28 (width) x 1.72 cm (height). The scanned area is therefore 2.20 $cm^2$ and the spatial resolution is better than 0.0125 mm/pixel (80 pixel/mm).

**Figure 1:** Images of a sample of beef *longissimus dorsi* muscle showing the development of pigment layers after 1 hour (left) and 8 hours (right) of exposure to air. The meat sample covers the upper 2/3 of each picture. The slightly curved line that delimits the end of the meat sample defines the surface in contact with air.

## Conclusions

Multispectral techniques can be used to study myoglobin layer development during meat oxygenation. It is an instrumental method, not biased by the observer or the illuminating source. It provides high spatial resolution and should permit in the future accurate analysis of pigment concentration distributions in meat. It is a non destructive method and pigment concentrations can be studied for long periods of time for the same sample.

## References


S.C. Seideman, H.R. Cross, G.C.Smith and P.R. Durland. "Factors associated with fresh meat color: a review". Journal of Food Quality, 6, 211-237 (1984).

M. Renerre (2004). Myoglobin oxidation and meat colour. VII Congreso Nacional de Color. Pamplona. Spain.

O'Keeffe, M. and Hood, D. E. (1982). Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability, Meat Science 7, 3, 209-228.

Powell, V. H., Macdougall, D.B. (1993). In: Proceedings of the 39th Congress Meat Science and Technology. Calgary (Canada), 141.

Gutzke, D.A., Trout, G.R. and D'Arcy, B.R. (1997). "The relationship between the colour stability and the formation of the metmyoglobin layer at the surface of beef, pork and venison muscles". In Proceedings 43rd International Congress of Meat Science and Technology (pp. 652-653) 27 July-1 August 1997. Auckland, New Zealand.

A.M.S.A. (1991). Guidelines for meat color evaluation. National Live Stock and Meat Board. 444 North Michigan Avenue. Chicago Illinois.